Thermo Scientific Appliskan®

User Manual

Rev. 1.2

Thermo SCIENTIFIC

Thermo Scientific Appliskan®

User Manual

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About This User Manual

Intended users

This user manual has been written for the actual user (e.g., laboratory technician) and provides information on the Thermo Scientific Appliskan, including the installation and operating instructions.

Read the manual in its entirety before operating the instrument.

How to use this user manual

This user manual has been designed to give you the information you need to:

- Review safety precautions
- Install the Appliskan
- Use the Appliskan in daily research use
- Perform basic cleaning and maintenance procedures
- Troubleshoot the instrument performance

This user manual also describes all the features and specifications of the Appliskan instrument. Refer to Chapter 6: “Technical Specifications”.

In Chapter 8: “Troubleshooting Guide” you will find explanations of all error messages and a problem-solving guide. The user should be familiar with the contents of Chapter 5: “Maintenance”.

For ordering information, refer to Chapter 9: “Ordering Information”.

For more information

For software-related issues, refer to the *Thermo Scientific SkanIt Software for Appliskan User Manual* (Cat. no. N05855). Both the user and software manuals can be found in PDF format on the SkanIt Software for Appliskan installation CD.

For the latest information on products and services, visit our worldwide websites on the Internet at:

`http://www.thermo.com`

`http://www.thermo.com/appliskan`

`http:/www.thermo.com/readingroom`

In an effort to produce useful and appropriate documentation, we appreciate your comments on this user manual to your local Thermo Fisher Scientific representative.

Safety symbols and markings

These symbols are intended to draw your attention to particularly important information and alert you to the presence of hazards as indicated.

Safety symbols and markings used on the Appliskan

The following symbols and markings appear on the type label and the instrument itself.

Power ON ▲

Power OFF ▲

Serial number ▲

Catalog number ▲

Date of manufacture ▲

Consult instructions for use ▲

Risk of radiation injury ▲

WEEE symbol This product is required to comply with the European Union's Waste Electrical & Electronic Equipment (WEEE) Directive 2002/96/EEC. ▲

A black label with the following text (Figure 2–3):

CAUTION: WARNING: DISCONNECT SUPPLY BEFORE SERVICING and AVERTISSEMENT: COUPER L'ALIMENTATION AVANT L'ENTRETIEN ET LE DEPANNAGE. ▲

Warning and other markings used in the documentation

The following symbols and markings appear in this user manual.

Warning Risk of electric shock. ▲

Warning Biohazard risk. ▲

Warning Risk of injury to the user(s). ▲

Warning Risk of ultraviolet radiation injury. ▲

Caution Risk of damage to the instrument, other equipment or loss of performance or function in a specific application. ▲

Note Marks a hint, important information that is useful in the optimum operation of the system, or an item of interest. ▲

Instrument safety and guidelines for use

1. Always follow basic safety precautions when using the Appliskan to reduce the risk of injury, biohazardous contamination, fire or electrical shock.

2. Read this user manual in its entirety prior to operating the instrument. Failure to read, understand and follow the instructions in the manual may result in damage to the instrument, injury to laboratory and operating personnel or poor instrument performance.

3. Observe all “Warning”, “Caution”, and “Note” statements as well as safety symbols and markings on the instrument and in the documentation.

4. Never open any other covers of the Appliskan than the dispenser cover (Figure 2–2) or emission and excitation/absorbance filter slide housing doors (Figure 2–2) while the instrument is plugged into a power source.

5. Never open any covers while the instrument is busy (when the LED indicator is orange).

6. The Appliskan is intended for laboratory research use only. Observe proper laboratory safety precautions, such as wearing protective clothing and following approved laboratory safety procedures. It is recommended that Good Laboratory Practices (GLP) are followed to guarantee reliable analyses.

7. Preventative maintenance instructions should be followed closely to keep the instrument in the best condition for maximum reliability. A poorly maintained instrument will not give the best results.

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Chapter 1
Introduction to the Appliskan

Overview

The Appliskan (Figure 1–1) is a filter-based multimode microplate reader with onboard shaker and incubator. It can also be equipped with up to two optional dispensers. The Appliskan is used to measure fluorescence intensity (FI), time-resolved fluorescence (TRF), fluorescence polarization (FP), absorbance and luminescence in endpoint and kinetic measurements in the UV/Vis/NIR range from appropriate 6 to 384-well microplate formats. Incubation can be carried out in a controlled incubation temperature up to 45°C. The instrument also allows shaking and reagent dispensing. The Appliskan is run on Thermo Scientific SkanIt Software for Appliskan 2.3 (or greater), which controls all the instrument functions and provides data processing as well as reporting functions.

Figure 1–1. Appliskan filter-based multimode microplate reader

Intended use

The Appliskan filter-based multimode microplate reader is intended for professional laboratory research use by trained personnel, who understand the nature of fluorometry, photometry and luminometry.

Use for self-testing is excluded.

The Appliskan is used to measure fluorescence intensity (FI), time-resolved fluorescence (TRF), fluorescence polarization (FP), absorbance and luminescence from appropriate 6 to 384-well plate formats defined

by Thermo Fisher Scientific in SkanIt Software. It also has incubation, shaking and reagent dispensing capabilities.

Refer to Chapter 6: “Technical Specifications”.

For validation of the entire system, it is recommended that Good Laboratory Practices (GLP) are followed to guarantee reliable analyses.

Advantages of using Appliskan

The Appliskan provides several advantages relating mainly to the principle of operation in that it:

- Supports applications requiring measurement in the UV/Vis/NIR wavelength range with all main detection technologies
- Enables measurement of multiple labels from the same well
- Allows optimization of the assays to different plate formats depending on the throughput requirements
- Enables incubation and shaking of samples
- Enables kinetic measurements due to dispensing and consecutive measurement
- Enables automation due to robot compatibility
- Is controlled by SkanIt Software for Appliskan that provides an easy and flexible assay setup and instrument control with an intuitive user interface, powerful data handling and report formatting capabilities

Chapter 2
Functional Description

Instrument layout

This section shows the front, internal and back views of the Appliskan instrument.

Front view

The front view of the Appliskan instrument and mains power supply box are shown in Figure 2–2. For more details on the mains power supply box, see Figure 2–4 A.

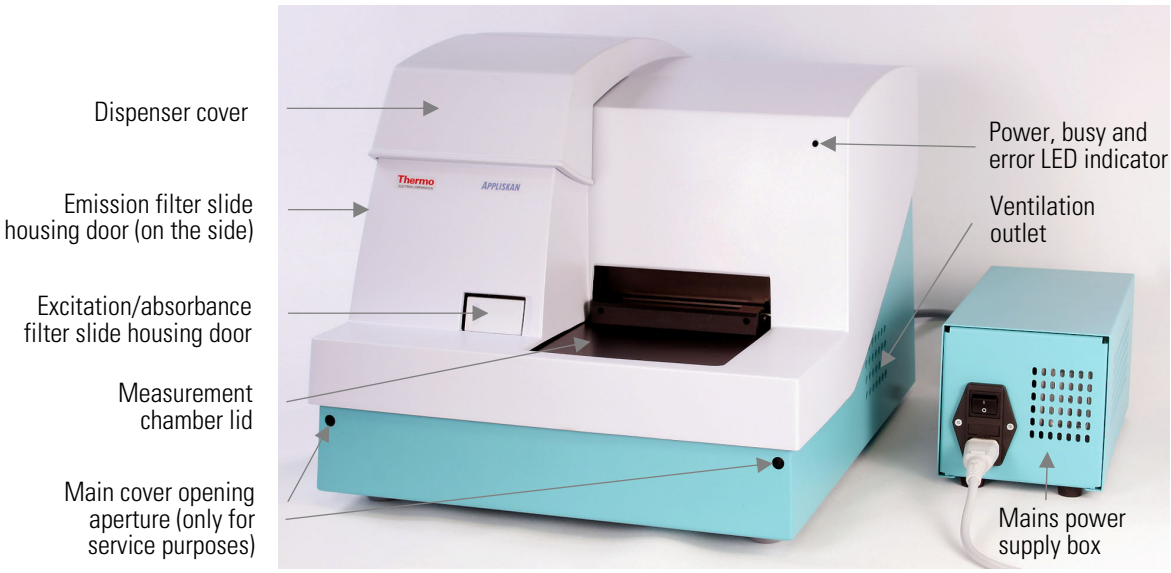


Figure 2–2. Appliskan front view

Back view

The back view of the Appliskan instrument is shown in Figure 2–3 and Figure 2–4 B.

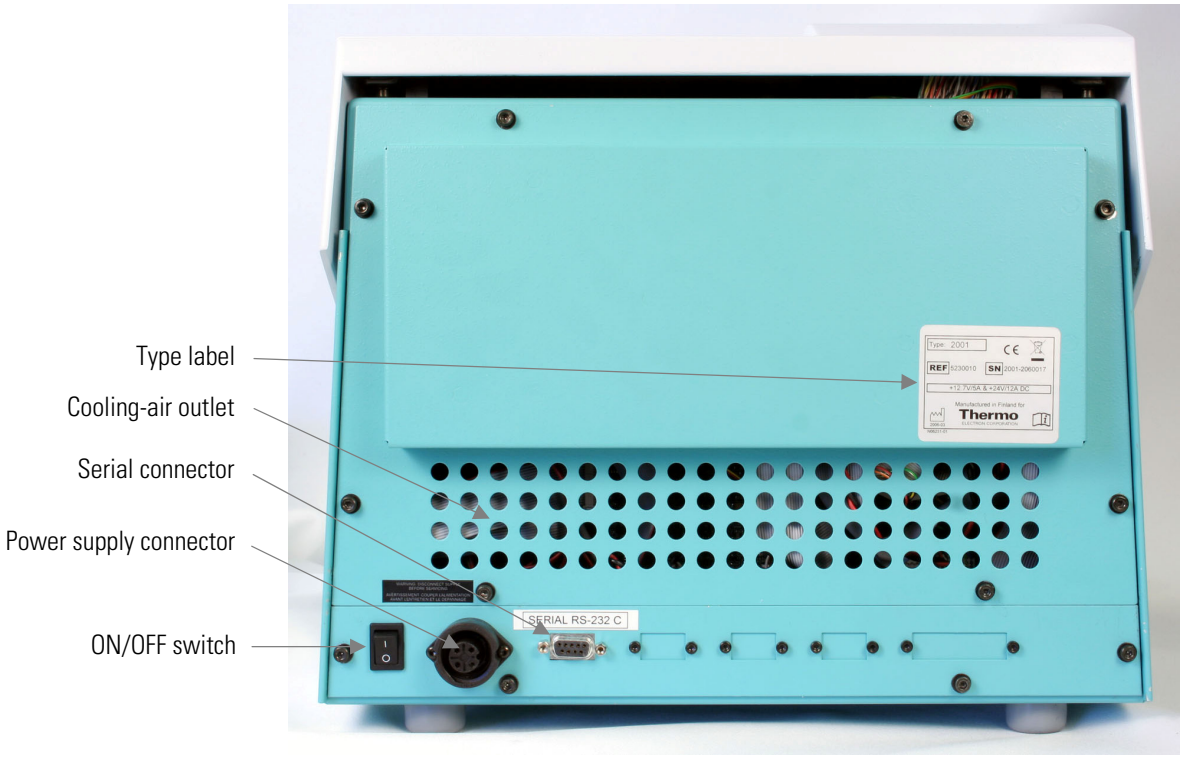


Figure 2–3. Appliskan back view

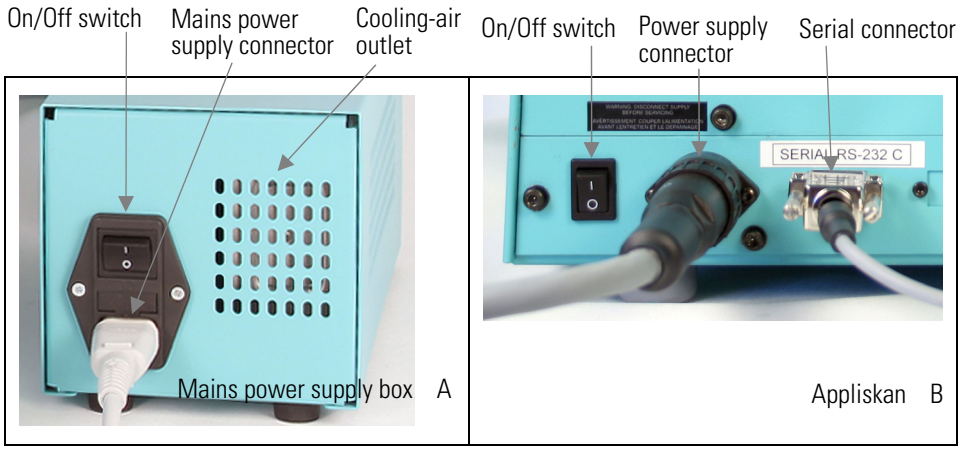


Figure 2–4. Close-up of the computer and mains and power supply connectors

Internal view

The internal view of the Appliskan instrument is shown in Figure 2–5.

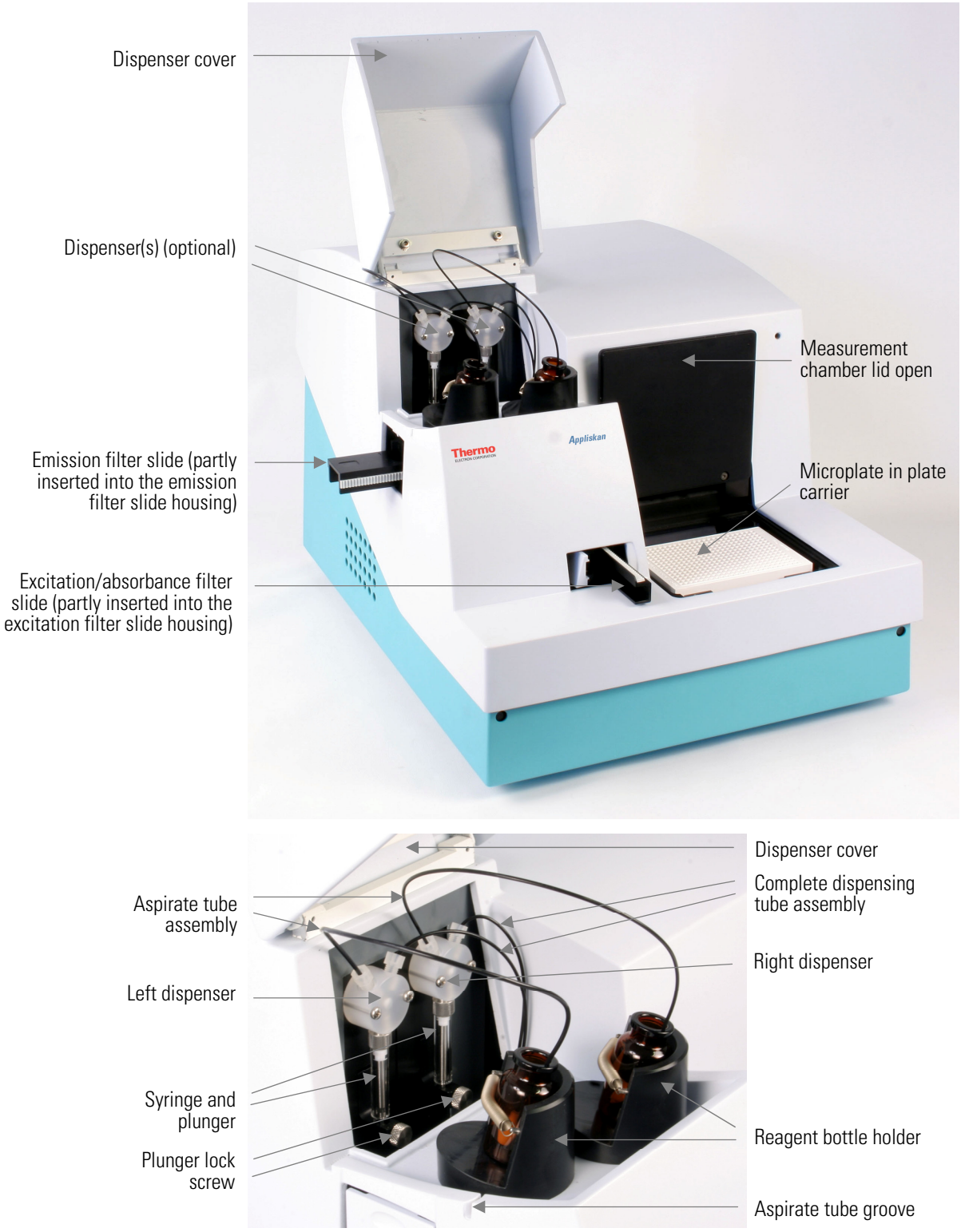


Figure 2–5. Appliskan internal views

Measurement techniques

This section describes the relevant measurement techniques, including fluorescence intensity (FI), time-resolved fluorescence (TRF), fluorescence polarization (FP), absorbance and luminescence.

Fluorescence intensity

Fluorescence is the phenomenon in which absorption of excitation light of a given wavelength by a fluorescent molecule is followed by the emission of light at longer wavelengths. Fluorescence intensity (FI) of the emitted light (RFU) at selected excitation and emission wavelengths is proportional to the concentration of the fluorescent molecule being investigated.

Fluorescent molecules have two characteristic spectra: an excitation spectrum which shows the wavelength-dependent amount of light absorbed and an emission spectrum which shows the wavelength-dependent amount of light emitted. No two compounds have exactly the same fluorescence spectra, thus making fluorometry a highly specific analytical technique.

Solid black plates are recommended for fluorescence intensity reads.

One of the major advantages of fluorescence detection is high sensitivity. This is important as relatively small changes in the assays can have significant physiological effects. In addition of fluorescence being a versatile tool in biochemistry, cell biology and molecular biology, it is also a powerful technique for studying molecular interactions in analytical chemistry, physiology, photochemistry and environmental science.

Fluorescence resonance energy transfer (FRET) is a fluorescence intensity based measurement technique. Two labels are required for FRET measurements: donor (fluorescent), and acceptor (either fluorescent or non-fluorescent). The emission spectrum of the donor needs to overlap with the absorption spectrum of the acceptor to allow the energy transfer to happen.

FRET allows homogeneous assay formats to be used in the detection of biological interactions. The change in the intensity of the generated FRET signal can be related to specific biological events, such as enzyme-mediated cleavage of DNA or protein substrates, protein-DNA interactions and protein-peptide interactions.

Time-resolved fluorescence

Time-resolved fluorescence (TRF) is a special form of fluorescence intensity where fluorescence lifetime of the signal is remarkably longer than in fluorescence intensity. TRF uses lanthanide labels which have similar excitation and emission spectra as fluorescence intensity labels. Every TRF label has a unique fluorescence lifetime parameter τ (tau) which reflects the duration of fluorescence emission after excitation has been switched off. In TRF measurements the lanthanide label is excited with light flashes and the resulting emission is detected after a label-specific delay time.

Typical biological samples have a fluorescence background with a very short lifetime, which has an effect on fluorescence intensity measurements. In TRF technology this biological background has decayed before the TRF signal is measured, giving improved assay performance.

Time-resolved fluorescence labels can well be used for resonance energy transfer applications as fluorescence intensity labels. This time-resolved fluorescence energy transfer technology is known as TR-FRET.

Solid white plates are recommended for time-resolved fluorescence reads. Solid black plates are more seldom recommended for some TR-FRET measurements, in accordance with the kit recommendation.

Fluorescence polarization

The concept of molecular movement and rotation is the basis of fluorescence polarization (FP). By using a fluorescent dye to label a small molecule, its binding to another molecule of greater size can be monitored through its speed of rotation. When the fluorescent dye is excited with plane-polarized light, tracers attached to molecules with high molecular weight emit a high level of polarized fluorescence since tracers are slower in rotations compared to tracers attached to smaller molecules. An increase in molecular volume of a fluorescent dye (due to binding) or a decrease (due to dissociation or enzymatic degradation) can be measured by FP. Fluorescence polarization therefore detects the binding of a tagged molecule to a target molecule.

Polarization (FP) value (in mP units), a dimensionless number, means the extent of molecular rotation during the period between excitation and emission. The measured polarization is a weighted average of the two values, thus providing a direct measure of the fraction of tracer bound to receptor and is defined by Equation 1.

$$mP = 1000 * \frac{I_S - I_P}{I_S + I_P}$$ **Equation 1**

Polarization values are inversely related to the speed of molecular rotation of the fluorescent target. FP increases as molecular weight or solvent viscosity increases. On the other hand, FP decreases as the excited state lifetime of the dye increases. Anisotrophy (FA) is another way to present polarization and is defined by Equation 2.

$$r = \frac{I_S - I_P}{I_S + 2I_P}$$ **Equation 2**

FP is a homogeneous technology and reactions are very rapid, taking seconds to minutes to reach equilibrium. The reagents are stable and large batches may be prepared. This results in high reproducibility. Because of these properties, FP has proven to be highly automatable, often performed with a single incubation with a single, premixed, tracer-receptor reagent. FP is easy and simple because it does not require immobilization or any washing steps, and it is used mainly in high-throughput screening in drug monitoring.

Solid black plates are recommended for FP reads.

FP applications include, for example, enzyme assays, receptor binding assays as well as protein-peptide and DNA-protein assays.

Absorbance

When a beam of light enters a sample, part of the light is absorbed by the sample and the rest is transmitted (passes through the sample).

Absorbance (A) is defined by Equation 1:

$$A = \log (I_o/I) \quad \textbf{Equation 1}$$

where: I_o = intensity of incident light

I = intensity of transmitted light

The absorbance is linearly related to the concentration of the absorbing compound by Bouguer-Lambert-Beer's Law (Equation 2).

$$A = \varepsilon C d \quad \textbf{Equation 2}$$

where: A = absorbance

ε = molar absorption coefficient [l/(mol*cm)]

C = concentration [mol/l]

d = pathlength [cm].

Solid clear, flat-bottom plates and clear, flat-bottom plates with white or black walls are recommended for absorbance reads.

Luminescence

Luminescence is the emission of light at visible wavelength by a substance. Luminescence is caused by the movement of electrons from more energetic states to less energetic states. In contrast to fluorometry, no excitation light is required. Luminescence can be caused by chemical or biochemical changes. The excitation energy is thus produced by a chemical reaction.

There are many types of luminescence that can be identified according to the source of energy which excites the emission. When the light energy emitted results from certain chemical reactions, chiefly oxidations, such as in the slow oxidation of phosphorus at ordinary temperatures, the emission is called *chemiluminescence*. When the luminescent chemical reaction occurs in a living system, such as in the glow of a firefly, the emission is called *bioluminescence*. Bioluminescence is luminescence produced by living organisms and is thought to be a type of chemiluminescence. Other examples of bioluminescence include glowworms, deep-sea organisms, and various fungi and bacteria found on rotting wood or decomposing flesh. Both luminescence types are detected by the instrument.

Solid white plates are recommended for luminescence reads.

Bioluminescence resonance energy transfer (BRET) is a non-destructive, cell-based assay technology that offers the ability to directly study complex protein-protein interactions in living cells. The assay is based on non-radiative energy transfer between fusion proteins containing a luciferase (bioluminescent donor) and a green fluorescent protein (GFP) mutant (fluorescent acceptor). Interactions between the two fusion proteins can bring the luciferase and GFP close enough for resonance energy transfer to occur, thus changing the color of the bioluminescent emission. The transfer efficiency depends on the degree of the spectral overlap, the relative orientation, and the distance between the donor and acceptor.

BRET is also a naturally occurring phenomenon in marine animals, such as the sea pansy *Renilla reniformis* and the jellyfish *Aequorea victoria*.

In most applications the fused donor is *Renilla* luciferase (Rluc) rather than aequorin, to avoid any intrinsic affinity for *Aequorea*-derived GFP mutant; the acceptor is the yellow fluorescent protein (YFP), to increase the spectral distinction between the two emissions.

Optical system

The Appliskan employs fluorometric, photometric and luminometric measurement techniques. Fluorometric and luminometric measurements are made from the top of the well and photometric measurements are made through the well.

The principle of the Appliskan optical measurement modules is shown in the following block diagram (Figure 2–6). Each submodule is described separately in the subsequent lower-level block diagrams (Figure 2–7 through Figure 2–11).

Principle of the optical system

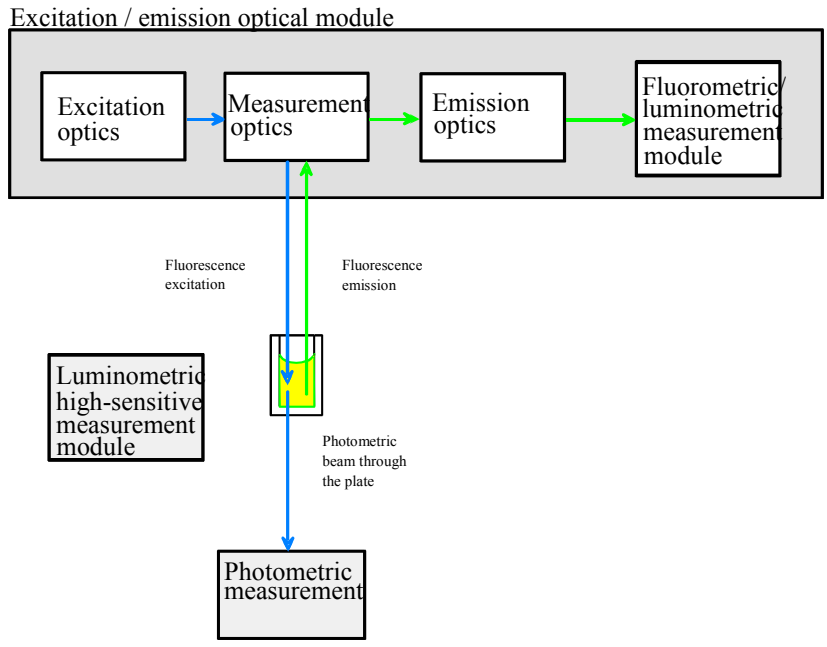


Figure 2–6. Appliskan optics

The Appliskan optical unit consists of four subunits (Figure 2–6):

- The excitation optics produces light of selected wavelength for fluorometric measurement and also for photometric measurement. Refer to “Excitation optics” on page 24.
- The measurement optics produces a high-definition optical beam for fluorometric and photometric measurements. The excitation light reference detector is incorporated into the measurement optics. Refer to “Measurement optics” on page 24.
- The emission optics carries out the reading of a selected wavelength for fluorometry and luminometry. Refer to “Emission reading module” on page 25.
- The photometric measurement module measures light-beam intensity passing through the well. Refer to “Photometric measurement module” on page 26.

Excitation optics

The excitation optics (Figure 2–7) consists of the light source and the wavelength selection devices.

Warning Do not open the optical covers under any circumstances. There is a risk of ultraviolet radiation injury.
Only authorized service personnel has permission to open the optical covers. ▲

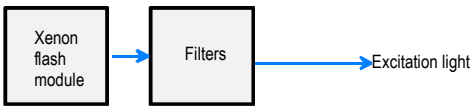


Figure 2–7. Excitation optics

Light source:

A xenon flash lamp is used as the light source. The lamp provides a wide spectral range needed for photometry and fluorometry. The lamp is pulsed at a 50 – 200 Hz rate and activated only when measuring. A short light pulse enables accurate TRF measurements.

One measurement consists of 1 to 500 flash pulses according to the measurement quality and measurement speed requirements.

Filters:

Excitation filters are used to block unwanted transmission. The filter is selected with SkanIt Software for Appliskan.

Measurement optics

The measurement optics module (Figure 2–8) is the front surface mirror optics system to generate a wavelength-independent, high-definition beam for fluorometric measurement and for photometric

measurement. Simultaneously the measurement optics collects emission light, which is fed to the emission reading channel.

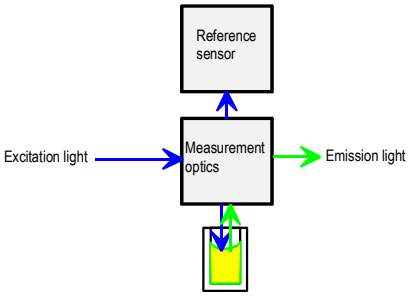


Figure 2–8. Measurement optics

The excitation beam intensity is measured by the reference sensor before the measurement beam enters the well. The reference sensor value is used to correct the result level to compensate for long-term and short-term flash intensity fluctuations.

Emission reading module

The emission optics (Figure 2–9) is basically similar to the excitation optics. Refer to “Excitation optics” on page 24.

Warning Do not open the optical covers under any circumstances. There is a risk of ultraviolet radiation injury. Only authorized service personnel has permission to open the optical covers. ▲

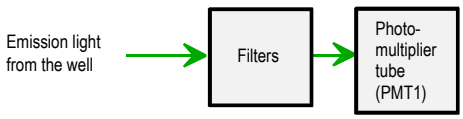


Figure 2–9. Emission optics of the standard mode

Emission optics is used in fluorometric and standard mode luminometric measurements (Figure 2–9), for example, BRET assays. Refer to “Luminescence on page 22.

Emission filters:

Emission filters are used to block unwanted transmission.

Emission detectors:

Emission light is converted into electrical signals by the photomultiplier tube (PMT1). The dynamic range is adjusted automatically. The high-sensitive mode has a separate PMT (PMT2) and no filters are used (Figure 2–10).

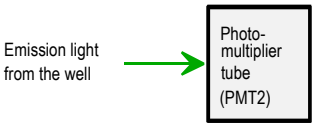


Figure 2–10. Emission optics of the high-sensitive mode

Photometric measurement module

Photometric measurement is carried out by using the excitation optics module as the photometric measurement light source.

The photometric measurement module (Figure 2–11) is just underneath the fluorometric measurement position.

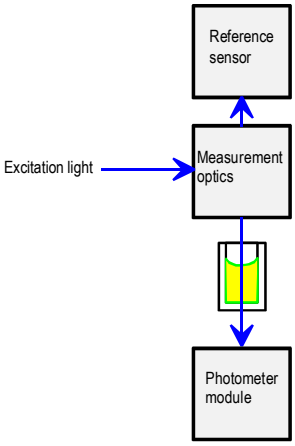


Figure 2–11. Photometric measurement module

Dispenser option

Up to two optional dispensers are available and are located in the instrument housing under the dispenser cover (Figure 2–5). The dispenser(s) is intended for accurate dispensing, in the range of 5 to 500 µl with increments of 1 µl. The dispenser(s) consists of a pump with a valve, a syringe (0.5 ml), tubing and a dispensing probe (Figure 2–12). Also a 1.0 ml syringe is available on request.

The instrument supports dispensing and consecutive reading, enabling fast signal monitoring of the reaction. The dispenser is located close to the dispensing position in order to achieve a low dead volume and minimal reagent consumption. This is important when using expensive reagents. Optimal design of the reagent bottle holder (Figure 2–5) also helps in using all the reagent.

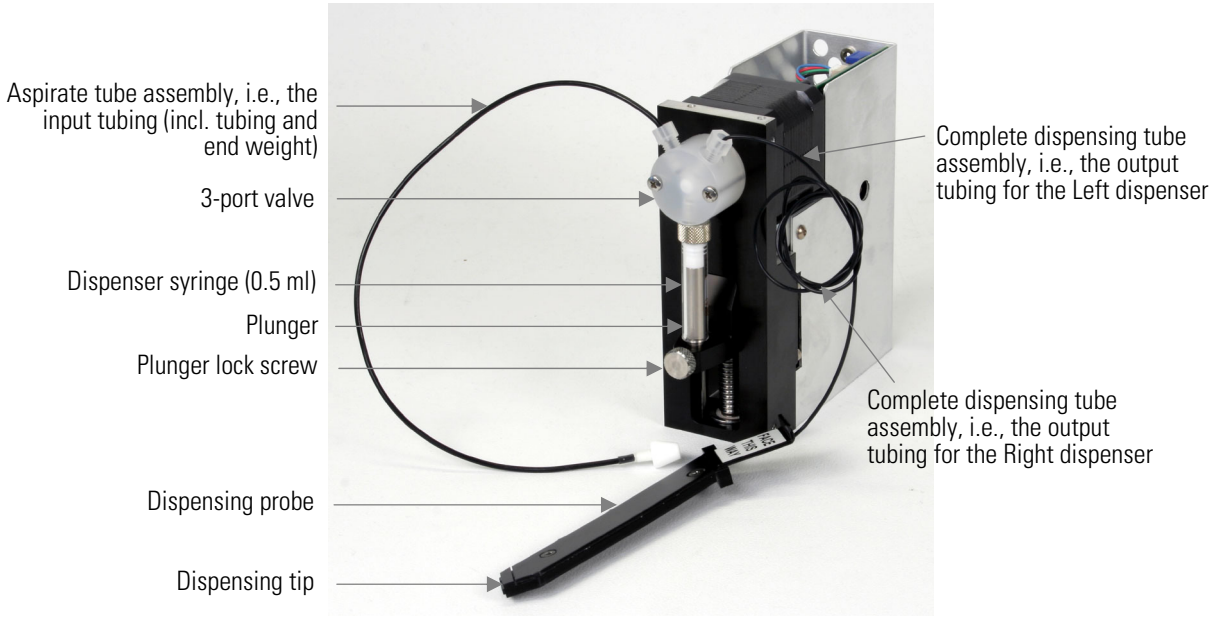


Figure 2–12. Appliskan dispensing system (unattached)

The dispensing probe, including the tubing and tip, is a complete set with either Left tubing or Left and Right tubing.

Refer to “How to set up the dispenser(s)” on page 33.

Incubator

The incubator is useful for temperature-critical applications, for example, certain enzyme assays and cell-based applications. It heats up to +45°C. The whole measurement chamber is heated for incubation purposes.

Plate carrier

The plate carrier has been specifically designed to obtain excellent measurement results for different plate formats.

Plate adapter

This section provides information on the plate adapter supplied with the instrument.

The adapter for SBS standard plates is shown in Figure 2–13. The adapter has to be inserted when SBS standard 96 and 384-well plates are used. Note that the maximum plate height is 20.5 mm without the adapter.

Refer to “How to install the plate adapter” on page 32.

Caution Note that the maximum total height of plates is manufacturer related. ▲

Refer to Figure 2–13 and Chapter 9: “Ordering Information”. For more information on plate type settings, refer to the *SkanIt Software for Appliskan User Manual* (Cat. no. N05855).

Figure 2–13. Adapter for SBS standard plates

Chapter 3
Installation

Installation checklist

This chapter on installation contains an outline of the points mentioned in the checklist below (Table 3–1).

Warning The Appliskan weighs about 27 kg [60 lbs.] and care must be taken when lifting it. Two persons must lift the instrument, one on each side, by hooking their fingers under the sides. ▲

Table 3–1. Installation checklist

Tick	Item
❑	Unpack the Appliskan instrument carefully. Refer to "How to unpack" on page 30. Keep the original packaging and packing material for future transportation.
❑	Check the delivery for completeness. Refer to "Checking delivery for completeness" on page 31.
❑	Check for damage during transport Refer to "Checking for damage during transport" on page 31.
❑	Place the instrument on a normal laboratory bench, taking into account both the environmental and technical prerequisites. Refer to "Environmental requirements" on page 31 and "Things to avoid" on page 31.
❑	Install the instrument. Refer to "Installation setups" on page 32. Install the adapter. Refer to "How to install the plate adapter" on page 32. Ensure that the aspirate tube assembly and the complete dispensing tube assembly are installed. Refer to "How to set up the dispenser(s)" on page 33.
❑	Connect the mains and power supply cables and the serial connector RS-232C. Refer to "How to ensure startup" on page 35.
❑	Install SkanIt Software for Appliskan. Refer to "How to install SkanIt Software for Appliskan" on page 35. Refer to the *SkanIt Software for Appliskan User Manual* (Cat. no. N05855).
❑	Perform the operational check. Refer to "Operational check" on page 36.

What to do upon delivery

This section covers the relevant procedures to be carried out upon arrival of the instrument.

How to unpack

Move the packed instrument to its site of operation. To prevent condensation, the instrument should be left in its protective plastic wrapping until the ambient temperature has been reached. Unpack the Appliskan instrument and accessories carefully with the arrows on the transport package pointing upwards. To remove the red transport foam insert inside the plate carrier, open the plate carrier first (Figure 3–14). Refer to the enclosed packing instructions.

Figure 3–14. Removing the transport foam insert

The following notes and instructions are sent with the instrument and are immediately available when you open the package:

- Packing instructions
- Packing list
- Warranty Certificate card
- Performance test reports
- *Appliskan User Manual* and *Quick Reference Guide*
- SkanIt Software for Appliskan package

Caution Do not touch or loosen any screws or parts other than those specifically designated in the instructions. Doing so might cause misalignment and will void the instrument warranty. ▲

Retain the original packaging and red transport foam insert for future transportation. The packaging is designed to assure safe transport and minimize transit damage. Use of alternative packaging materials may

invalidate the warranty. Also retain all instrument-related documentation provided by the manufacturer for future use.

If you relocate your instrument or ship it for service, refer to "How to pack for service" on page 77.

Checking delivery for completeness

Check the enclosed packing list against order. If any parts are missing, contact your local Thermo Fisher Scientific representative or Thermo Fisher Scientific Oy.

Checking for damage during transport

Visually inspect the transport package, the instrument and the accessories for any possible transport damage.

If the carton has been damaged in transit, it is particularly important that you retain it for inspection by the carrier in case there has also been damage to the instrument.

If any parts are damaged, contact your local Thermo Fisher Scientific representative or Thermo Fisher Scientific Oy.

Environmental requirements

When you set up your Appliskan, avoid sites of operation with excess dust, vibrations, strong magnetic fields, direct sunlight, draft, excessive moisture or large temperature fluctuations.

- Make sure the working area is flat, dry, clean and vibration-proof and leave additional room for cables, covers, and so on.
- Make sure the ambient air is clean and free of corrosive vapors, smoke and dust.
- Make sure the ambient temperature range is between +10°C (50°F) and +40°C (104°F).
- Make sure relative humidity is between 10% and 80% (non-condensing).

The Appliskan does not produce operating noise at a level that would be harmful. No sound level measurements are required after installation.

Caution Do not operate the instrument in an environment where potentially damaging liquids or gases are present. ▲

Things to avoid

Do not smoke, eat or drink while using the Appliskan. Wash your hands thoroughly after handling test fluids. Observe normal laboratory procedures for handling potentially dangerous samples. Use proper protective clothing. Use disposable gloves. Ensure that the working area is well ventilated.

Never spill fluids in or on the equipment.

Technical prerequisites

Place the instrument on a normal laboratory bench. The net weight of the unit is about 27 kg [60 lbs.].

Warning The Appliskan must be lifted with care. Two persons must lift the instrument, one on each side, by hooking their fingers under the sides. ▲

The instrument operates at voltages of 100 — 240 Vac and the frequency range 50/60 Hz.

Installation setups

This section describes the installation setups that have to be carried out before instrument operation.

How to install the plate adapter

The black adapter for SBS standard plates (Figure 2–13) is easy to install. Refer to “Plate adapter” on page 27.

To install the plate adapter, follow these steps:

1. Always insert the plate adapter into the plate carrier when you are measuring SBS standard 96 or 384-well plates (Figure 3–15). It fits in either side since it is symmetrical. Use the rectangular finger cavities to easily insert the plate adapter.

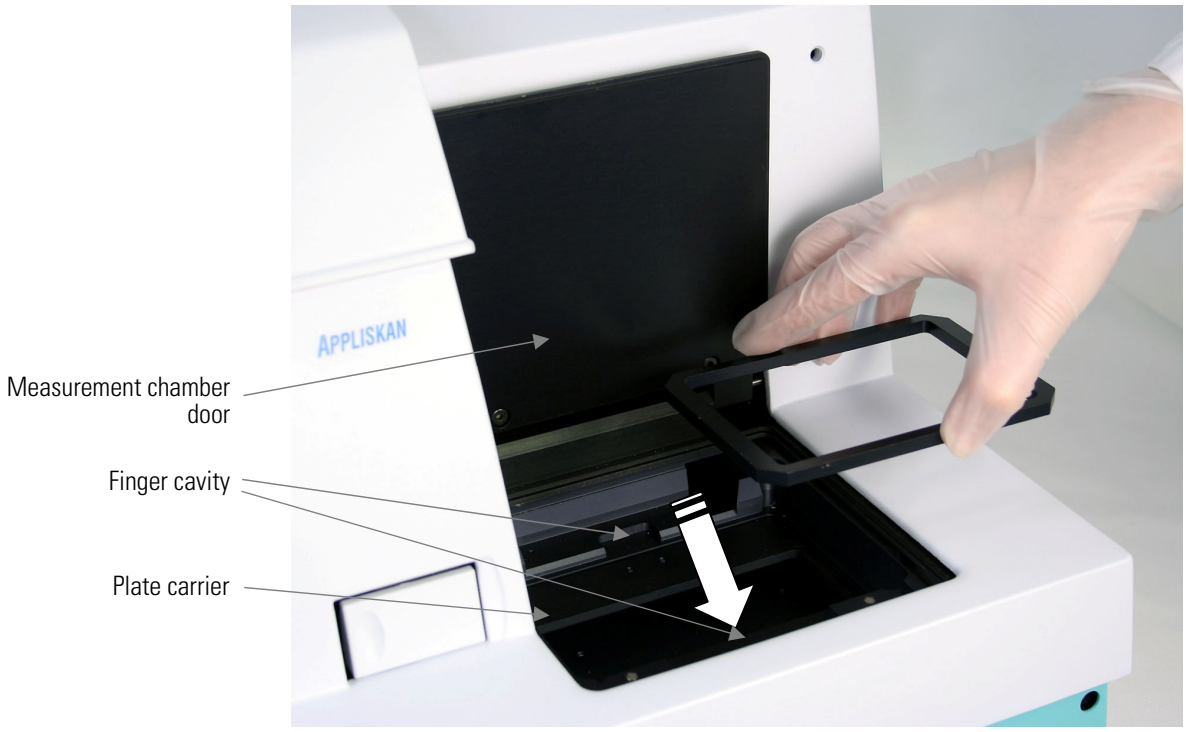


Figure 3–15. Inserting or removing the plate adapter

2. Ensure that you remove the plate adapter when measuring other than SBS standard plates. Use the rectangular finger cavities to easily remove the adapter.

How to set up the dispenser(s)

Up to two optional dispensers may be present in the instrument. The dispenser(s) is factory installed and located on the left-hand side of the instrument under the dispenser cover (Figure 3–16).

Caution If the dispenser(s) is not properly installed, leakage may occur. ▲

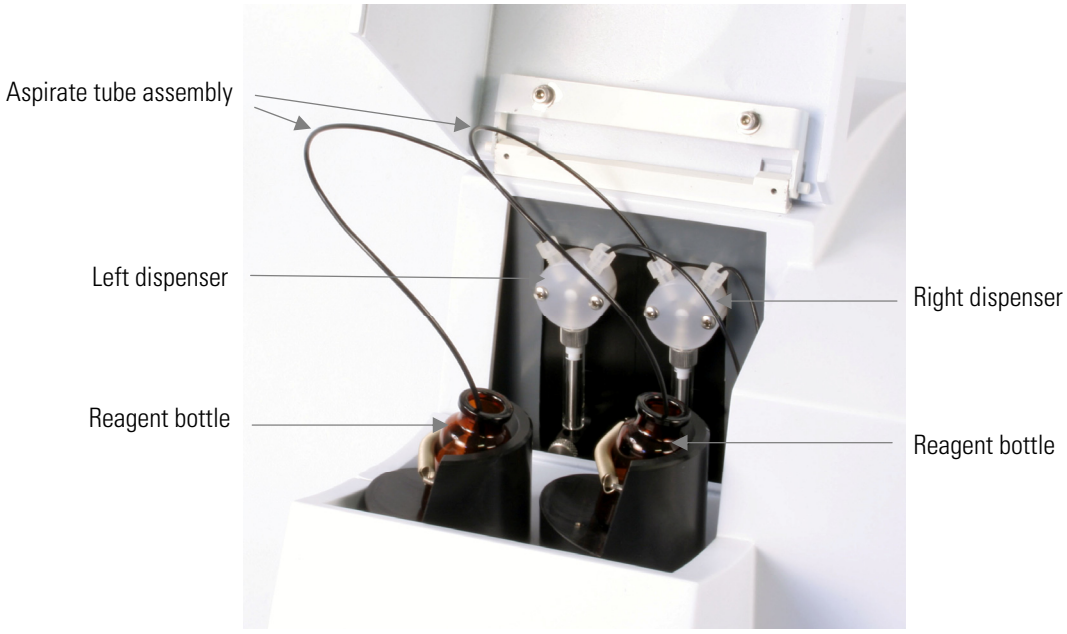


Figure 3–16. Appliskan with the dispenser cover open

1. The aspirate tube assembly is packed with the accessories.

   Fit the aspirate tubing (Figure 3–18) into the left hole of the valve. Ensure that the aspirate tubing is finger tight. The aspirate tubing is used to fill the syringe with reagent. When using the dispenser, make sure the aspiration tube end is completely submerged in the contents of the reagent bottle and there is a sufficient volume of the reagent in the bottle (for all priming and actual dispensing).

   If you have a larger reagent bottle beside the instrument, the aspirate tube can be inserted into the aspirate tube groove (Figure 2–5). Also a longer aspirate tube is available on request.

2. The complete dispensing tube assembly is factory installed.

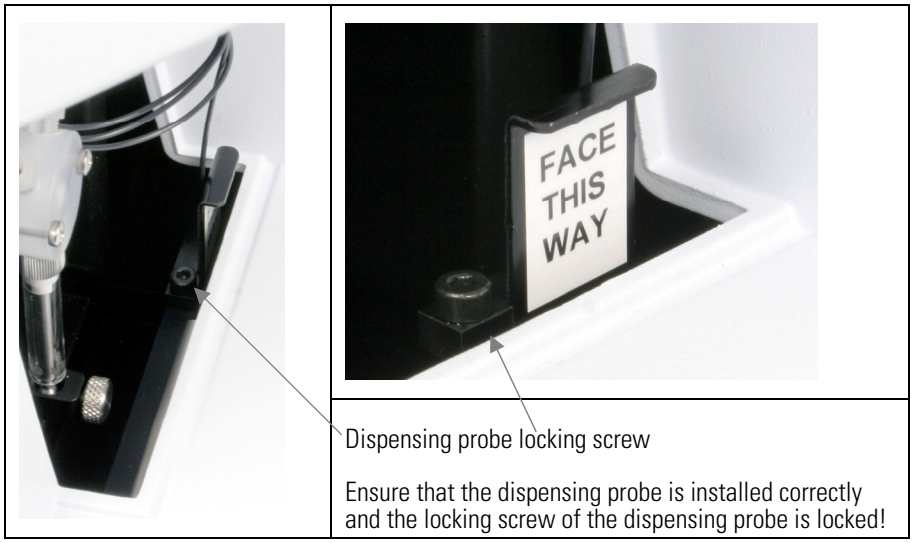


Figure 3–17. Locking screw of the dispensing probe fitted

Ensure that the complete dispensing tube assembly (Figure 3–18) is inserted into the right hole of the valve and is finger tight. The dispensing tube is used to dispense reagent from the syringe into a microplate.

3. Ensure that the dispensing probe is inserted into the dispensing probe slot with one or two tubings, depending on the amount of dispensers (Figure 4–27).

4. Otherwise, fit the locking screw of the dispensing probe with the hexagonal screwdriver supplied (Figure 3–17).

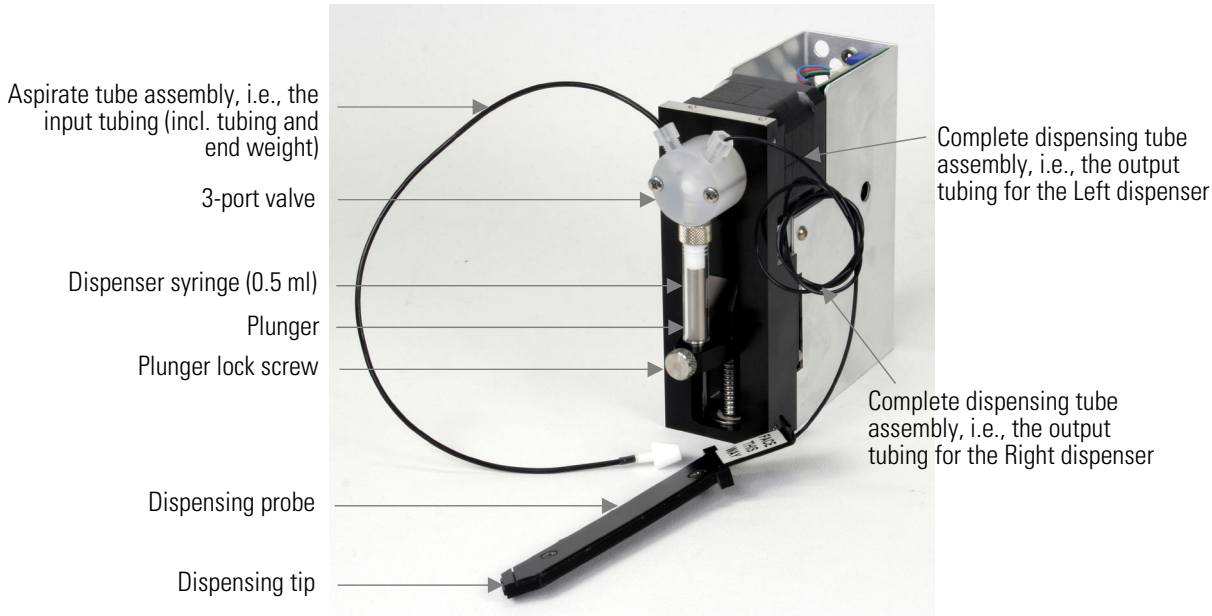


Figure 3–18. Dispenser assembly (unattached)

How to ensure startup

This section shows the location of all relevant connectors and how to connect the mains and power supply cables.

Warning Ensure that both the mains power switch in front of the mains power supply box (Figure 3–19 B) as well as the power switch on the right-hand side of the instrument back panel are in the OFF position (Figure 3–19 A). Never operate your instrument from a power outlet that has no ground connection. ▲

1. First connect the fixed power supply cable of the mains power supply box to the power supply connector (Figure 3–19 A) on the right-hand side of the back panel of the Appliskan.

2. Then connect the RS-232C serial connector on the back panel of the Appliskan (Figure 3–19 A).

3. Connect the serial cable to the computer. Refer to the computer requirements in “General specifications” on page 81. Also refer to the corresponding computer manual.

4. Finally connect the mains power supply cable to the mains power supply connector in front of the mains power supply box (Figure 3–19 B) and connect it to a correctly installed line power outlet that has a protective conductor that is grounded.

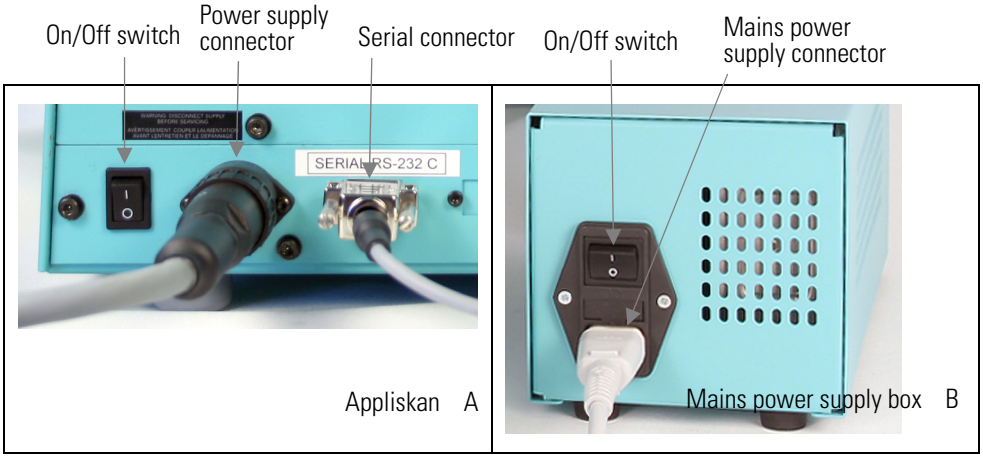


Figure 3–19. Connecting the mains and power supply connectors

How to install SkanIt Software for Appliskan

Refer to the *SkanIt Software for Appliskan User Manual* (Cat. no. N05855) for installing SkanIt Software for Appliskan.

Note Operate the instrument only with software and hardware specifically designed or selected for it. Thermo Fisher Scientific assumes no liability for the use of third-party software applications. ▲

Operational check

First switch the mains supply power box (Figure 3–19 A) ON and then the Appliskan ON (Figure 3–19 A).

The instrument has a sophisticated control system. The instrument automatically performs a complete set of initialization tests and adjustments. The mechanical, electrical and optical functions of the instrument are checked at startup, for example:

- plate carrier positioning
- excitation/absorbance and emission filter slides
- non-volatile memory
- temperature measurement electronics
- measurement electronics
- xenon flash lamp
- reference detector
- dispenser(s)

When the initialization tests and adjustments have been successfully completed, the LED indicator (Figure 2–2) turns from orange to green. Refer to “Switching on” on page 39.

If anything fails in the initialization tests or adjustments, the LED indicator will turn red. The user will thus be informed of the error through SkanIt Software for Appliskan. In this case, try switching the instrument OFF and ON again. If the failure is repeated, contact authorized technical service. Refer to “Error messages” on page 91.

The instrument also performs automatic signal long-time stability checks during runtime.

After startup the instrument is ready for operation. Since the instrument calibrates itself, you can start measuring immediately as soon as the instrument has been turned on. However, the stabilization of the incubator can take up to 30 minutes. It is further recommended to carry out an empty run to verify proper instrument operation.

Chapter 4
Routine Operation

The operation of the Appliskan filter-based multimode microplate reader is controlled by an external computer and run on SkanIt Software for Appliskan.

Note Operate the instrument only with software and hardware specifically designed or selected for it. Thermo Fisher Scientific assumes no liability for the use of third-party software applications. ▲

Note It is recommended that the assay includes internal quality controls. ▲

Do's and Don'ts of the Appliskan

This section on Do's and Don'ts summarizes all the relevant procedures on what to do and what not to do.

Do

- In case of any emergencies occurring during operation, switch off and unplug the instrument immediately. Carry out corrective measures. If the corrective measures taken do not help, contact authorized technical service.
- Carry out the operational check before normal use.
- Ensure that you select a correct plate type. Too high a plate may get jammed and with too low a plate the dispensing might fail and pass by. Note that the maximum height of a plate is 20.5 mm without the adapter.
- When placing a microplate into the plate carrier, always make sure that the correct plate type has been selected in SkanIt Software for Appliskan (Protocol Properties: **Plate template**) before you do anything else.
- Ensure that the plate type, adapter and the SkanIt Software for Appliskan plate template match.
- Ensure that the adapter has been inserted into the plate carrier when SBS standard 96 and 384-well plates are used.
- Keep the plate adapter clean.
- Ensure that the bottom of each microplate is dry. Fluid on the bottom of a microplate may present a contamination hazard.

- Take into account the chemical resistance of the dispenser(s) (Table 4–3) and microplates. Use organic solvents in the dispenser(s) with caution.
- Make sure you do not dispense into the instrument by mistake. Ensure that a correct microplate has been inserted and that the microplate is not too full.
- Prime the dispenser(s) by discarding the liquid into an external waste container. Hold on to the dispensing probe while priming.
- Wipe off any drops on the dispensing tip after priming or washing before insertion of the dispensing probe into the dispensing probe slot.
- Handle the filter slides with outmost care.
- Always leave an empty microplate in the plate carrier at shutdown.
- Check the installation and maintenance checklists.

Don't

- Use for self-testing is excluded.
- Do not use the instrument if it appears that it does not function properly.
- Do not touch or loosen any screws or parts other than those specifically designated in the instructions. Doing so might cause misalignment and will invalidate the instrument warranty.
- Do not open the optical covers under any circumstances. There is a risk of ultraviolet radiation injury. Only authorized service personnel has permission to open the optical covers.
- Never open any other cover of the Appliskan than the dispenser cover (Figure 2–2) or the emission and excitation/absorbance filter slide housing doors (Figure 2–2) while the instrument is plugged into a power source.
- Do not open any covers while the instrument is busy (when the LED indicator is orange).
- Do not open the measurement chamber lid during measurement.
- Do not use plates with dimensions exceeding the top rim of the plate carrier. Note that the maximum total height of plates is manufacturer related.
- Never touch the surfaces of the excitation/absorbance and emission filters. Clean the filters with lens cleaning solution or isopropyl alcohol and a lintfree lens tissue.
- Do not screw a filter at a slant. The filter must be straight. Otherwise the light will diffract in a different way.

- Do not change the orientation of the polarization film if you have to change a polarization filter under the film in a filter slide.
- Excitation and emission filters must not overlap!
- Do not, for example, throw the filter slides about, otherwise the filters may easily break.
- Do not force the filter slide(s) in, since the toothed edge can be damaged. Insert only up to the position where it stops with about 1.5 cm of the filter slide outside the filter slide housing.
- Never spill fluids in or on the equipment. Wipe up all spills immediately.
- Never use any liquids that can cause any precipitation or clotting or that contain any mechanical particles with the automatic dispenser(s).
- Do not use formaldehyde under any circumstances.
- Do not spill any alkalines onto any instrument surfaces to avoid damage of the instrument. If needed, use suitable protection covering.

Switching on

The Appliskan is equipped with a mains power supply box (Figure 2–2), a power switch (ON/OFF) (Figure 2–3) and a three-color LED indicator (Figure 2–2). First switch the mains power supply box on from the front left-hand ON/OFF switch (Figure 3–19 B). Then immediately switch the Appliskan on by pressing the power switch on the bottom right-hand side of the instrument back panel into the ON position (Figure 3–19 A). Note that it takes a short while before the instrument itself is turned on.

When the instrument is switched ON, the color indicates the state of the instrument (Table 4–2).

Table 4–2. Front panel indicator light

LED		Instrument status
Green		The instrument is ready and waiting for a command from SkanIt Software for Appliskan.
Orange		The instrument is busy, executing startup functions or commands from SkanIt Software for Appliskan.
Red		The instrument has found an error. The error message is sent to SkanIt Software for Appliskan to be acknowledged by the user.

Warning Never operate your instrument from a power outlet that has no ground connection. ▲

Loading the microplate

This section describes what issues to take into account when loading a microplate.

Caution Ensure that you select a correct plate type. Too high a plate may get jammed and with too low a plate the dispensing might fail and pass by. The maximum plate height is 20.5 mm without the adapter. ▲

Caution When placing a microplate into the plate carrier, always make sure the correct plate type has been selected in SkanIt Software for Appliskan (Protocol Properties: **Plate template**) before you do anything else. ▲

To insert the microplate, follow these steps:

1. Ensure that the plate type and the SkanIt Software for Appliskan plate template match.

2. If the measurement chamber lid is closed, first open the measurement chamber lid by selecting either Execute > **Run Plate Out** or clicking the button on the toolbar in SkanIt Software for Appliskan.

Figure 4–20. Microplate loaded

3. Ensure that the black adapter for SBS standard 96 and 384-well plates (Figure 2–13) is inserted when measuring plates lower than 15 mm. Refer to “How to install the plate adapter” on page 32.

4. Load the microplate onto the instrument plate carrier for measurement (Figure 4–20). Always insert the microplate so that the A1 corner is positioned in the top left corner of the plate carrier (Figure 4–20).

   The plate carrier is able to handle microplates of slightly different sizes, therefore, the free space in the plate carrier is to a certain extent larger than, for example, the standard 96-well plate. Insert the plate with the front edge first. The left and right positioning levers in the plate carrier will then automatically position the plate correctly and securely into the upper left A1 corner of the plate carrier when the measurement chamber lid is closed. The left positioning lever will position the plate correctly in the vertical direction.

5. After this the protocol is executed with SkanIt Software for Appliskan. The software operates according to the selected protocol parameters. Refer to the *SkanIt Software for Appliskan User Manual* (Cat. no. N05855).

How to install or remove the filter slides

There are two types of filter slides (Figure 4–22, Figure 4–23 and Figure 5–34), smaller excitation filter slides for 12.5 mm excitation or absorbance filters, and larger emission filter slides for 25 mm emission filters, each with five hard coded slides from A to E with IDs available.

Refer to Chapter 9: “Ordering Information”. Refer also to “How to handle the filter slides” on page 61.

Caution Do not touch the surfaces of the excitation/absorbance and emission filters. ▲

To *install* the excitation/absorbance or emission filter slide, follow these steps:

1. Open the excitation/absorbance or emission filter slide housing door (Figure 4–21). First press the finger notch on the door and then push the door in to the left for the excitation/absorbance filter slide housing door and up for the emission filter slide housing door.

2. Insert the *excitation/absorbance filter slide* with the white toothed edge facing up and the ID of the filter slide to the right (Figure 4–22).
   Insert the *emission filter slide* with the toothed edge facing you and with the filters up (Figure 4–23). Ensure that the filter slides are inserted far enough with only some 1.5 cm of the slide outside the housing.

Caution Insert only up to the position where it stops. Do not force the filter slide in beyond this point, since the toothed edge can be damaged. ▲

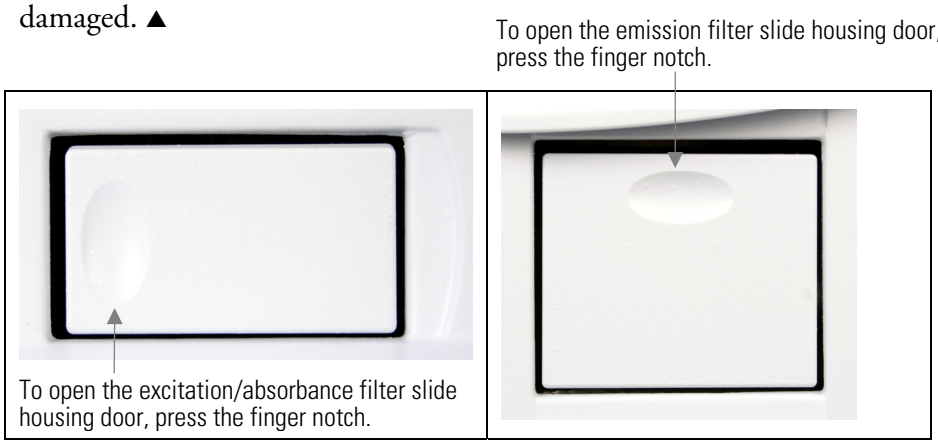


Figure 4–21. Opening the filter slide housing doors

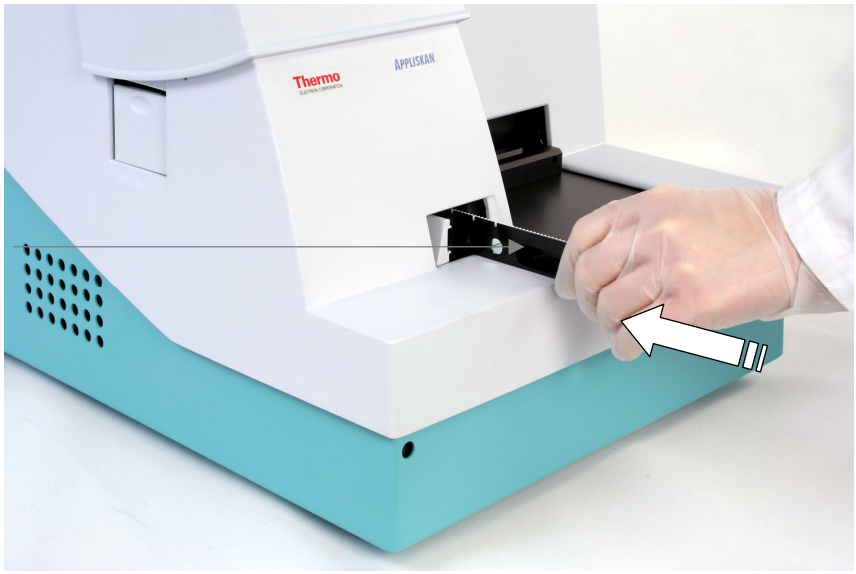


Figure 4–22. Inserting the excitation/absorbance filter slide

Figure 4–23. Inserting the emission filter slide

3. The motor controls the movements of the filter slides, which in turn are primarily controlled by SkanIt Software for Appliskan. Select Execute > **Run Excitation Slide In** or **Run Emission Slide In** in SkanIt Software for Appliskan to drive the filter slide in. The entire filter slide will then be inserted.

4. Close the excitation/absorbance or emission filter slide housing door manually by pulling it out using the finger indention cavity as aid (Figure 4–24).

To *remove* the excitation/absorbance or emission filter slide, follow these steps:

1. Select Execute > **Run Excitation Slide Out** or **Run Emission Slide Out** in SkanIt Software for Appliskan. The excitation/absorbance or emission filter slide housing door opens automatically and the filter slide slides partly out.

2. Pull the filter slide out towards you holding on to the elongated metal edges.

3. Close the excitation/absorbance or emission filter slide housing door manually by pulling it out using the finger indention cavity as aid (Figure 4–24).

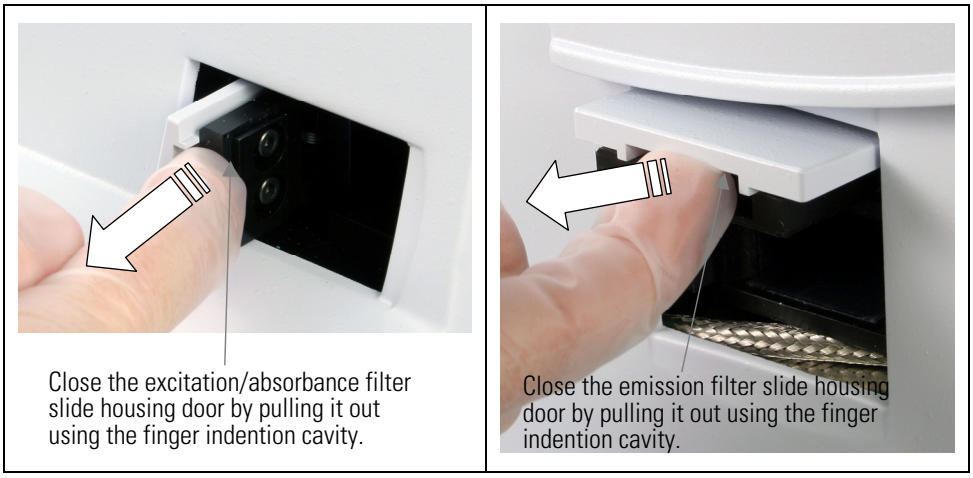


Figure 4–24. Closing the filter slide housing doors

Fluorometric measurement

This section explains the measurement processes in fluorescence intensity (FI), time-resolved fluorescence (TRF) and fluorescence polarization (FP) measurements. SkanIt Software for Appliskan controls the measurement processes. Refer to the *SkanIt Software for Appliskan User Manual* (Cat. no. N05855).

Fluorescence intensity

In fluorescence intensity (FI), the following actions are carried out by the instrument:

1. The measurement chamber lid is closed.

2. Excitation and emission filters are selected by driving the excitation and emission filter slides.

3. The dynamic range is automatically adjusted.

4. The wells are measured with a selected measurement time that can vary from 10 to 1000 ms in fluorescence intensity measurements. There is one xenon lamp flash for each 10 ms period of measurement time.

   The amount of xenon lamp flashes affects the quality of the measurement result. Thus, the more flashes, the better the quality of the result. The measurement time can be set to 10 to 1000 ms for fluorescence intensity measurements.

   It is recommended to measure using a 500 ms measurement time in fluorescence intensity measurements, which normally produces good results. If there is a necessity to improve the quality of the results, the flash amount should be increased.

   The result is the mean value of individual 10 ms readings during the total measurement time.

   The lamp is pulsed at a 100 Hz rate and activated only when measuring.

Time-resolved fluorescence

In time-resolved fluorescence (TRF) measurements, the following actions are carried out by the instrument:

1. The measurement chamber lid is closed.

2. Excitation and emission filters are selected by driving the excitation and emission filter slides.

3. The dynamic range is automatically adjusted.

4. The wells are measured with a selected measurement time that can vary from 5 to 10000 ms in time-resolved fluorescence (TRF) measurements. There is one xenon lamp flash for each 5 ms period of measurement time.

The amount of xenon lamp flashes affects the quality of the measurement result. Thus, the more flashes, the better the quality of the result. The measurement time can be set to 5 to 10000 ms for TRF measurements.

It is recommended to measure using a 500 ms measurement time in TRF measurements, which normally produces good results. If there is a necessity to improve the quality of the results, the flash amount should be increased.

The result is the mean value of individual 5 ms readings during the total measurement time.

With TRF measurements there are two additional user-defined measurement parameters: TRF delay time and TRF integration time. The TRF delay time defines the time difference between the excitation flash and emission signal collection, while the TRF integration time defines the time used for emission signal collection. When the Appliskan performs a TRF measurement, it excites the sample with a very short light pulse, waits for the defined TRF delay time and then collects the signal during the defined TRF integration time. These actions form one TRF measurement cycle (Figure 4–25) that is performed within a 5 ms period. The cycle is repeated as many times as defined by the measurement time.

The lamp is pulsed at a 200 Hz rate and activated only when measuring.

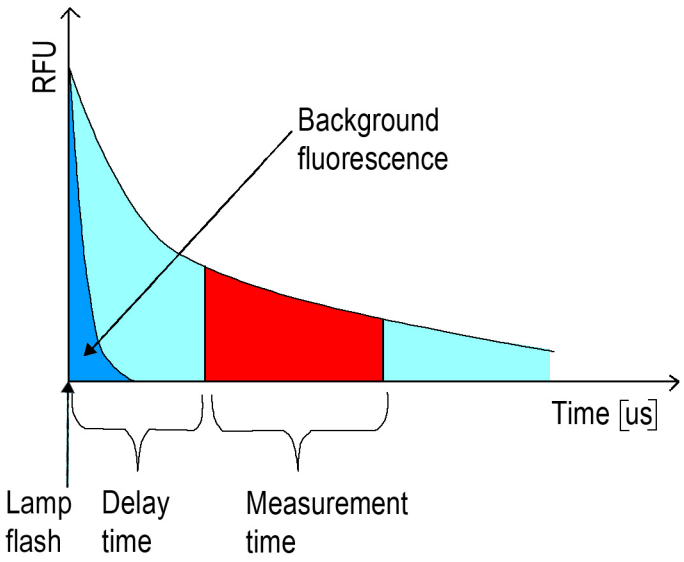


Figure 4–25. Structure of the TRF measurement cycle

Caution Do not open the measurement chamber lid during measurement. ▲

Caution Because of the relative nature of fluorometry, it is recommended to use known samples or controls to verify instrument operation. ▲

Fluorescence polarization

In fluorescence polarization (FP) measurements, the following actions are carried out by the instrument:

1. The measurement chamber lid is closed.

2. The polarization, excitation and *first* emission filters are selected by driving the excitation and emission filter slides. And then the same polarization, excitation and *second* emission filters are selected by driving the excitation and emission filter slides.

3. The wells are repeatedly measured with both direction polarizators with a selected measurement time that can vary from 10 to 10000 ms. It is recommended to measure using a 500 ms measurement time (default), which produces good results. The lamp is pulsed at a 100 Hz rate and activated only when measuring.

Photometric measurement

SkanIt Software for Appliskan controls the measurement processes. Refer to the *SkanIt Software for Appliskan User Manual* (Cat. no. N05855).

In absorbance (Abs) measurements, the following actions are carried out by the instrument:

1. The measurement chamber lid is closed.

2. The measurement filter slide is selected by driving the excitation/absorbance filter slide.

3. In the photometric calibration procedure the instrument reads the air blank level. In long measurement procedures calibration is performed in a suitable phase without disturbing the measurement timing.

4. The wells are measured with a selected measurement time that can vary from 20 to 1000 ms. There is one xenon lamp flash for each 20 ms period of measurement time.

5. The amount of xenon lamp flashes affects the quality of the signal. Thus, the more flashes, the better the quality of the result. The measurement time can be set to 20 to 1000 ms.

It is recommended to measure using a 200 ms measurement time (default), which produces good results. If there is a necessity to improve the quality of the results, the flash amount should be increased.

The result is the mean value of the number of 20 ms readings during the total measurement time. Longer than 200 ms measurement times are recommended to reduce noise if the measured absorbance level is high.

The lamp is pulsed at a 50 Hz rate and activated only when measuring.

Caution Do not open the measurement chamber lid during measurement. ▲

Luminometric measurement

SkanIt Software for Appliskan controls the measurement processes. Refer to the *SkanIt Software for Appliskan User Manual* (Cat. no. N05855).

High-sensitive mode

In high-sensitive mode luminescence measurements, the following actions are carried out by the instrument:

1. The measurement chamber lid is closed.
2. The wells are measured with a selected measurement time that can vary from 10 to 10000 ms. The default measurement time is 1000 ms.
3. The measurement time affects the quality of the signal. The longer the time, the better the quality of the result.

Note When high-sensitive mode is used in the measurement, the emission filter slide must be in. ▲

Standard mode

In standard mode luminescence measurements, the following actions are carried out by the instrument:

1. The measurement chamber lid is closed.
2. The filter is selected by driving the emission filter slide.
3. The wells are measured with a selected measurement time that can vary from 10 to 10000 ms. The default measurement time is 1000 ms.

4. The measurement time affects the quality of the signal. The longer the time, the better the quality of the result.

Note When standard mode is used in the measurement, the emission filter slide must be in. If no filter is selected for your session, one position of the filter slide must be empty. ▲

Other functions

The Appliskan also has shaking, incubating and reagent dispensing capabilities, which are presented below.

Shaking

SkanIt Software for Appliskan controls the shaking processes. Refer to the *SkanIt Software for Appliskan User Manual* (Cat. no. N05855).

The linear mechanical shaking function is used for shaking the microplate in order to mix the samples. Movement of the track mechanism can perform the shaking action.

The shaking amplitude can be set to 1 to 10 mm.

Caution Some combinations of speed and plate type may cause too high centrifugal forces inside the well area, resulting in spills inside the measurement chamber. ▲

Incubating

SkanIt Software for Appliskan controls the incubation processes. Refer to the *SkanIt Software for Appliskan User Manual* (Cat. no. N05855).

Incubation can be carried out with 6 to 384-well plates. The incubation allows certain enzyme assays, cellular assays and other temperature-critical applications to be read under controlled conditions. The incubator heats up to 45°C. Refer to “Incubator” on page 27.

The whole measurement chamber is heated for incubation purposes.

Note The samples in the microplate reach the target temperature usually much later than the instrument. ▲

Dispensing

This section provides information on how to use and maintain the optional dispenser(s). SkanIt Software for Appliskan controls the dispensing processes. Refer to the *SkanIt Software for Appliskan User Manual* (Cat. no. N05855).

The Appliskan can be equipped with one or two optional dispensers (Figure 2–5), allowing dispensing from 5 to 500 µl in 1 µl increments.

Caution SkanIt Software for Appliskan checks that priming has been done before the dispenser(s) is used. ▲

Caution Make sure you do not dispense into the instrument by mistake. Ensure that:

- A correct microplate has been inserted.
- The microplate is not too full. ▲

The assembly includes external pumps and reagent bottles which are connected to the dispensers inside the Appliskan instrument. Small reagent bottles are held in available reagent bottle holders (Figure 3–16). Big reagent bottles fit beside the Appliskan.

Protocols allow you to specify the use of one or both dispensers. They are identified by the names Left and Right (or L and R) even when only one dispenser is connected. Also specified in the protocols are the delays required to allow completion of dispensing. In the dispensers used, the speed is Low, Medium, High or User defined (%). The dispensing probe has a fixed dispensing tip with one or two tubing outlets depending on the amount of dispensers present that discharge(s) during dispensing into the well (Figure 3–18). The dispensing occurs according to a stroke per well. When the dispenser(s) is not in use, the dispensing probe should be stored in the dispensing probe slot after priming or dispenser washing (Figure 4–27).

Flash type luminescent assays, for example, require the use of dispensers to provide reagents that initiate the reagent reaction in the wells. The addition of reagents and the plate reading require precise coordination. Use of the dispensers requires that the fluid path is maintained and free of contamination. The measurement, which allows highly sensitive detection, takes place < 1 s after dispensing and involves the movement of the plate carrier from the dispensing position to the measurement position.

Also a 1.0 ml syringe is available on request.

Priming

The Appliskan can be primed using SkanIt Software for Appliskan. Prime the dispenser tubing, if necessary. Either select Execute > **Prime…** or press the  button on the toolbar in SkanIt Software for Appliskan.

To prime, follow these steps:

Caution For safety reasons a plate must be present on the plate carrier during priming. Insert an empty plate of the same type as the actual assay plate into the plate carrier. This plate is used as a waste plate in case of the dispensing probe being located in the dispensing probe slot during priming. ▲

1. Remove the locking screw of the dispensing probe (Figure 4–26).

Figure 4–26. Loosening the locking screw

2. Lift the dispensing probe from the dispensing probe slot (Figure 4–27).

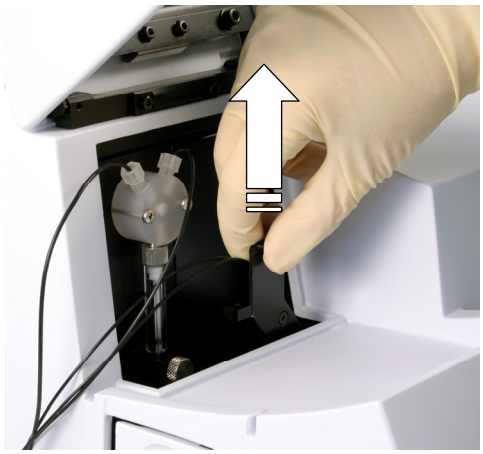


Figure 4–27. Removing the dispensing probe from its slot

3. Prime the dispenser(s) by discarding the liquid into an external waste container. Hold on to the dispensing probe while priming (Figure 4–28).

Figure 4–28. Priming into a waste container

4. Prime until the tubings are completely filled with fluid. Visually check that the dispensing jet is uniform and straight.

5. Wipe off any drops on the dispensing tip before insertion. Insert the dispensing probe into the dispensing probe slot after priming.

Caution Ensure that the dispensing probe is installed correctly and the locking screw of the dispensing probe is locked (Figure 4–29). ▲

Figure 4–29. Replacing the locking screw of the dispensing probe

6. Replace the locking screw of the dispensing probe (Figure 4–29).

Refer to the priming instructions (Execute > **Prime…**) in the *SkanIt Software for Appliskan User Manual* (Cat. no. N05855). The minimum priming volume is 600 µl and the recommended volume 800 µl (default).

Emptying

To avoid waste of reagents, you may wish to empty reagent that is in the tubing back into the bottles. This may well be the case if expensive reagents are used.

To empty, follow these steps:

1. The aspirate tube can remain in the reagent bottle.

2. Enter an estimate (800 µl) in µl of the volume of reagent remaining in the tubing.

3. If you do not wish to empty, close the **Empty** dialog.

Refer to the emptying instructions (Execute > **Empty…**) in the *SkanIt Software for Appliskan User Manual* (Cat. no. N05855).

Dispenser rinsing

The dispenser(s) of the Appliskan can be rinsed using SkanIt Software for Appliskan. For more information, refer to the *SkanIt Software for Appliskan User Manual* (Cat. no. N05855).

To rinse the dispenser, follow these steps:

1. Unfasten the dispensing probe locking screw and remove the dispensing probe from the dispensing probe slot (Figure 4–26 and Figure 4–27).

2. Ensure that you have emptied the tubings. Refer to “Emptying” on page 52.

3. Remove the aspirate tube assembly from the reagent container. It is recommended to rinse the aspirate tube assembly with deionized distilled water.

4. Place the aspirate tube in clean deionized distilled water.

5. Hold the dispensing probe over the waste container and prime with a large volume, for example, 5000 µl. Select the **Prime** command (Execute > **Prime…**) through SkanIt Software for Appliskan.

6. Empty the tubings with the **Empty** command (Execute > **Empty…**) through SkanIt Software for Appliskan.

7. Lift the aspirate tube assembly from the deionized distilled water container.

8. Wipe off any drops from the tubings.

9. Replace the dispensing probe into the dispensing probe slot (Figure 4–27 and Figure 3–17).

10. Replace the aspirate tube assembly into a clean and empty vessel.

Dispensing and measurement

The instrument does not support simultaneous dispensing and reading.

For more information, refer to the *SkanIt Software for Appliskan User Manual* (Cat. no. N05855).

Caution Never use any liquids that can cause any precipitation or clotting or that contain any mechanical particles with the automatic dispenser(s). ▲

You may need to adjust the dispensing speed. The default setting is for water. You can find the adjustments and selections in SkanIt Software for Appliskan.

When dispensing is started, the liquid volume in the well should be less than half of the total volume (for example, the volume should be less than 200 µl in a typical 96-well plate).

Helpful hints

To maintain dispenser performance, keep the following in mind when operating the dispensing module(s):

- Wipe up all spills immediately.
- Dispensing cold fluids may cause leaks, due to differing coefficients of expansion of Teflon and glass. Leaks may occur when dispensing fluids that are at or below 15°C (61°F).
- Use organic solvents in the dispenser(s) with caution. Using organic solvents may reduce tubing and seal life. Refer to “Chemical resistance of the dispenser(s)” on page 54.

Chemical resistance of the dispenser(s)

Table 4–3 provides guidelines for compatibility with materials used in the fluid path of the dispenser(s). Compatibility information is based on charts provided by the material manufacturer. It is recommended that each laboratory determines compatibility for their respective applications.

Caution Failure to determine compatibility of chemicals used in individual applications with the dispenser(s) may result in damage to the dispenser(s) and/or test results. ▲

Plastic materials used in the dispenser(s):

Teflon (PTFE, TFE, FEP): tubing, valve plug and seal

Kel F: valve body

Polypropylene (PP): fittings for tubing

Note Kel F is the brand name for 3M's PCTFE, that is, polychlorotrifluoroethylene. The present brand name is Neoflon CTFE, manufactured by Deikon. ▲

Caution Also take into account the chemical resistance of microplates. ▲

Classification in the table:

—	No data available
0	No effect — excellent
1	Minor effect — good
2	Moderate effect — fair
3	Severe effect — not recommended
*	Polypropylene — satisfactory to 22°C (72°F)
**	Polypropylene — satisfactory to 49°C (120°F)

Table 4–3. Compatibility chart of solvents suitable with the plastic materials used in the dispenser(s)

Solvent	Teflon	Kel F	Polypropylene
Acetaldehyde	0	0	0
Acetates	—	0	0
Acetic acid	0	0	0
Acetic anhydride	—	0	—
Acetone	0	0	0
Acetyl bromide	0	—	—
Ammonia	0	—	0
Ammonium acetate	0	—	—
Ammonium hydroxide	0	0	0
Ammonium phosphate	—	0	0
Ammonium sulfate	—	0	0
Amyl acetate	0	—	3
Aniline	0	0	0
Benzene	0	3	*
Benzyl alcohol	0	0	0
Boric acid	0	0	0
Bromine	0	0	*
Butyl alcohol	0	0	1
Butyl acetate	0	—	*
Carbon sulfide	0	—	*
Carbon tetrachloride	0	1	3
Chloroacetic acid	0	0	—
Chlorine	0	1	3
Chlorobenzene	—	—	3
Chloroform	0	—	3
Chromic acid	0	0	—
Cresol	0	—	*
Cyclohexane	0	—	3
Dimethyl sulfoxide (DMSO)	0	0	0
Ethers	0	—	**
Ethyl acetate	0	—	0
Ethyl alcohol	0	—	0
Ethyl chromide	0	1	3
Formaldehyde	0	0	0
Formic acid	0	0	0

Continued

Cont.

Solvent	Teflon	Kel F	Polypropylene
Freon	0	2	0
Gasoline	0	0	3
Glycerin	0	0	0
Hydrochloric acid	0	0	0
Hydrochloric acid (conc.)	0	0	0
Hydrofluoric acid	0	0	*
Hydrogen peroxide	0	0	0
Hydrogen peroxide (conc.)	0	0	0
Hydrogen sulfide	0	0	0
Kerosene	0	0	0
Methyl ethyl ketone (MEK)	0	—	0
Methyl alcohol	0	—	0
Methylene chloride	0	0	3
Naphtha	0	1	0
Nitric acid	0	0	0
Nitric acid (conc.)	0	0	—
Nitrobenzene	0	—	**
Phenol	0	—	0
Pyridine	0	—	—
Silver nitrate	0	—	0
Soap solutions	0	—	0
Stearic acid	0	—	*
Sulfuric acid	0	0	0
Sulfuric acid (conc.)	0	0	—
Sulfurous acid	0	0	0
Tannic acid	0	0	0
Tanning extracts	—	—	—
Tartaric acid	0	—	—
Toluene	0	1	**
Trichloroethylene	0	3	3
Turpentine	0	0	**
Water	0	0	0
Xylene	0	0	*

Shutdown

To shut down the Appliskan, follow these steps:

Warning Leave an empty microplate in the instrument as a safety precaution if a dispenser(s) is present in the instrument. ▲

1. Rinse the dispenser tubing(s) (Figure 3–18) out thoroughly with distilled water after each use.

Warning Remove any used plates still in the instrument. Dispose of all microplates as biohazardous waste. ▲

2. If the plate carrier is not dirty, close the measurement chamber lid by selecting either Execute > **Run Plate In** or clicking the  button on the toolbar in SkanIt Software for Appliskan.

3. First switch the Appliskan off by pressing the power switch on the bottom right-hand side of the instrument back panel into the OFF position (Figure 3–19 A). Then switch the mains power supply box off on the front left-hand side into the OFF position (Figure 3–19 B). Note that it takes a short while before the mains power supply box is closed.

   Switch the instrument off after daily use or at least if it is not in use for prolonged periods of time, for example, over a weekend.

4. However, if the plate carrier is dirty, first switch the Appliskan off by pressing the power switch on the bottom right-hand side of the instrument back panel into the OFF position (Figure 3–19 A).

5. Then wipe the plate carrier surface and the adjacent instrument surface, for example, the dispensing area under the dispenser cover (Figure 3–16), with a soft cloth or tissue paper moistened with distilled water, a mild detergent (SDS, sodium dodecyl sulfate) solution.

6. If you have spilt infectious agents on the instrument, disinfect with 70% ethanol or some other disinfectant (see “Decontamination procedure” on page 75).

7. After cleaning or disinfection close the measurement chamber lid through SkanIt Software for Appliskan.

Emergency situations

In case there is any abnormal situation during operation, such as fluids spilling inside the instrument, follow these steps:

1. Switch OFF the instrument (Figure 3–19 A).

2. Unplug the instrument immediately from the power supply (Figure 3–19 A) and the mains power supply box (Figure 3–19 B).

3. Carry out appropriate corrective measures. However, do not disassemble the instrument.

4. If the corrective measures taken do not help, contact authorized technical service or your local Thermo Fisher Scientific representative.

Chapter 5
Maintenance

Maintenance checklist

This chapter on maintenance contains an outline of the points mentioned in the checklist below (Table 5–4). Contact local authorized technical service or your local Thermo Fisher Scientific representative for assistance, if necessary.

Table 5–4. Maintenance checklist

Item	Daily	Weekly	Monthly	Yearly
Keep the instrument free of dust. See "Regular and preventive maintenance" on page 60.	✓			
Wipe away any solutions from outer surfaces immediately to prevent damage, and wipe with deionized distilled water. See "Regular and preventive maintenance" on page 60.	✓			
If any surfaces have been contaminated with biohazardous material, disinfect with a mild sterilizing solution. See "Regular and preventive maintenance" on page 60.	✓			
Clean the case of the instrument periodically. See "Regular and preventive maintenance" on page 60.		✓		
Clean the plate carrier when necessary. See "How to clean the plate carrier" on page 61.		✓		
Clean the dispensing area when necessary. See "How to clean the dispensing area" on page 61.	✓			
Maintain the dispenser(s). See "Routine maintenance of the dispenser(s)" on page 62.	✓	✓		
Clean the filters when necessary. See "Cleaning the filters" on page 62.	✓	✓		
Maintain the filters. See "Visual filter check" on page 61.				✓
Replace the dispenser input and output tubing when necessary. See "Replacing the aspirate tube assembly or the complete dispensing tube assembly" on page 65.			✓	✓
Replace the dispenser syringe, if necessary. See "Replacing a dispenser syringe" on page 65.				✓
Replace the 3-port valve, if necessary. See "Replacing the 3-port valve" on page 67.				✓
Replace the filters in the filter slides, if necessary. See "Replacing the filters in the filter slides" on page 68.		✓	✓	
Ensure proper shutdown. See "Shutdown" on page 57.	✓	✓		
Decontaminate the instrument when relocating the instrument or sending it for service. See "Decontamination procedure" on page 75.				✓
Service the instrument regularly. See "Regular and preventive maintenance" on page 60 and "Service contracts" on page 78.				✓

✓ = depending on the laboratory conditions and the use of the instrument

Regular and preventive maintenance

For reliable daily operation, keep the instrument free of dust and liquid spills.

It is recommended to clean the case of the instrument periodically to maintain its good appearance. A soft cloth dampened in a warm, mild detergent solution will be sufficient.

Caution Abrasive cleaning agents are not recommended, because they are likely to damage the paint finish. ▲

Caution Painted surfaces can be cleaned with most laboratory detergents. Dilute the cleaning agent as recommended by the manufacturer. Do not expose painted surfaces to concentrated acids, alkalines or alcohols for prolonged periods of time as damage may occur. ▲

Plastic covers and surfaces can be cleaned with a mild laboratory detergent or ethanol.

It is recommended to service the instrument at least yearly. Refer to “Service contracts” on page 78.

If you believe that liquid has entered the Appliskan, first switch the instrument (Figure 3–19 A) and then the mains power supply box off (Figure 3–19 B) and unplug the instrument. Carry out corrective measures. Refer to “Decontamination procedure” on page 75 for aid. If necessary, contact your local Thermo Fisher Scientific representative or the Thermo Fisher Scientific technical service department. Refer to “How to pack for service” on page 77 and “Service request protocol” on page 96.

Although the Appliskan is constructed from high-quality materials, you must immediately wipe away spilt saline solutions, solvents, acids or alkaline solutions from outer surfaces to prevent damage.

Caution If local or laboratory regulations prescribe regular decontamination, it is not advisable to use formaldehyde, since even small traces of formaldehyde negatively affect the enzyme being used in EIA tests resulting in bad test results. ▲

Warning If any surfaces have been contaminated with biohazardous material, a mild sterilizing solution should be used. ▲

Caution Do not autoclave any part of this instrument. ▲

Caution Do not use alkaline or chlorite solutions for cleaning any parts of the measurement chamber, which may result in immediate damage to the instrument. ▲

How to clean the plate carrier

To clean the plate carrier, follow the instructions below.

Caution Keep the plate carrier clean to avoid dust and dirt from entering the measurement chamber. Clean the plate carrier surface (Figure 3–15), including the adapter (Figure 2–13), at least once a week using a soft cloth or tissue paper soaked in a mild detergent solution or 70% ethanol. Wipe up spills immediately. Do not use formaldehyde. ▲

If you have spilt infectious agents on the plate carrier, decontaminate according to "Decontamination procedure" on page 75.

Warning Ensure that the bottom of each microplate is dry. Fluid on the bottom of a microplate may present a contamination hazard. Use proper laboratory practices when handling any hazardous materials. ▲

Caution Keep the plate adapter clean (Figure 2–13). ▲

How to clean the dispensing area

To clean the dispensing area, follow the instructions below.

Caution Keep the dispensing area under the dispenser cover (Figure 3–16) clean. Clean the surface daily using a soft cloth or tissue paper soaked in a mild detergent solution, soap solution or ethanol. Wipe up spills immediately. Do not use formaldehyde. ▲

Warning If any surfaces have been contaminated with biohazardous material, a mild sterilizing solution should be used. ▲

How to handle the filter slides

Hold the excitation/absorbance and emission filter slides from the elongated metal edges according to Figure 4–23 and Figure 5–34.

Caution

- Never touch the surfaces of the excitation/absorbance or emission filters.
- Handle the filter slides with outmost care. Do not, for example, throw the filter slides about, otherwise the filters may easily break.
- Keep the filter slides protected from dirt and dust. ▲

Visual filter check

The quality of the filter(s) determines the dynamic range of the instrument. The useful life of a filter depends on environmental factors, such as dust, humidity and temperature. Filters have a one-year warranty.

To carry out the visual check, follow these steps:

1. Visually check the filter(s) by holding it against an even light source. If the color is even, then the filter is suitable for use.

2. If the filter appears to be mottled or discolored, discard the filter since it is either damaged or defective.

3. Use a spectrophotometer as the best alternative to measure the filters.

Cleaning the filters

To clean the filter(s), follow these steps:

Warning Always wear gloves when performing any kind of maintenance or service, especially if it involves potential contact with spilled fluids or fluid residues of any kind. ▲

Caution Never touch the surfaces of the interference filters or optical lenses.

Use of organic solvents, such as dichloromethane, may cause harm to the optics in the microplate reader. Extreme caution is advised when using organic solvents. Always use a plate lid and avoid placing a plate containing these materials in the microplate reader for prolonged periods of time. Damage caused by the use of incompatible or aggressive solvents is not covered by the instrument warranty. ▲

1. Clean the filter(s) with lens cleaning solution or isopropyl alcohol and a lintfree lens tissue.

Caution Do not use any other liquids to clean the filters. Avoid any harsh treatment. ▲

2. Wipe the filter(s) with a lint-free cloth, for example, a microfiber cloth, or a lens tissue.

3. Store the filter(s) either in a filter slide or in a storage facility designed for it.

Routine maintenance of the dispenser(s)

To obtain optimal performance and maximum useful life for the optional dispenser(s) (Figure 3–16), it is important that the recommended cleaning maintenance instructions are followed. Refer also to “Dispenser rinsing” on page 52.

The Appliskan is a very sensitive instrument. Therefore, take special care to avoid any contamination of any parts of the dispenser tubing and follow all Good Laboratory Practices recommendations.

Daily maintenance

The basic maintenance procedure should be performed regularly and on a daily basis to ensure proper dispenser operation.

1. Rinse the dispenser tubing (Figure 5–30) out thoroughly with distilled water after each use. Leave the fluid pathway filled for storage.

2. Inspect the dispenser(s) for leaks, and correct any problems immediately.

3. Wipe up all spills on and around the dispenser(s) immediately.

4. Do not allow the dispenser(s) to run dry for more than a few cycles.

Weekly maintenance

Clean the fluid path thoroughly on a weekly basis to remove precipitates such as salts, eliminate bacterial growth, and so on, using one of the procedures outlined below. There are three agents with which the dispenser(s) may be cleaned:

- Weak detergent
- 10% bleach (for example, sodium hypochlorite)
- Weak base and acid

Weak detergent or 10% bleach

Remove the dispensing probe from the dispensing probe slot (Figure 4–26 and Figure 4–27) and do not let any cleaning fluids enter the measurement chamber. Use external containers.

To clean the dispenser(s) (Figure 3–16) with weak detergent or 10% bleach, follow these steps:

1. Prime the dispenser(s) with a weak detergent solution or a 10% bleach solution. Make a solution of 10% bleach by adding one part of commercial bleach to nine parts of water. Leave the solution in the dispenser(s) with the syringe (Figure 5–30) fully lowered for 30 minutes.

2. After the 30-minute period, remove the aspirate tubing (Figure 5–30) from the detergent or bleach solution and remove all the fluid from the syringe and tubing into a waste container.

3. Prime the dispenser(s) a minimum of 10 cycles with distilled or deionized water. Leave the fluid pathways filled for storage.

Weak base and acid in sequence

Remove the dispensing probe from the dispensing probe slot (Figure 4–26 and Figure 4–27), and do not let any cleaning fluids enter the measurement chamber. Use external containers.

To clean the dispenser(s) (Figure 3–16) with weak base and acid, follow these steps:

1. Prime the dispenser(s) with 0.1 M NaOH and leave the solution in the dispenser(s) for 10 minutes with the syringe (Figure 5–30) fully lowered.

Caution Do not spill any 0.1 M NaOH onto any instrument surfaces to avoid damage of the instrument. If needed, use suitable protection covering. ▲

2. Flush the dispenser(s) with distilled or deionized water.

3. Prime the dispenser(s) with 0.1 M HCl, and leave the solution in the dispenser(s) for 10 minutes with the syringe fully lowered.

4. After the 10-minute period, remove the aspirate tubing (Figure 5–30) from the 0.1 M HCl solution, and remove all the fluid from the syringe and tubing into a waste container.

5. Prime the dispenser(s) a minimum of 10 cycles with distilled or deionized water.

Periodic maintenance

There are three parts which require periodic maintenance: tubing, syringe and valve. If they become worn out, you are likely to notice these symptoms:

- Poor precision and accuracy
- Air bubbles
- Leakage
- Drops and spills

The frequency of replacement will depend on the duty cycle, fluids used and instrument maintenance.

If any of these symptoms occur and it is not obvious which component is causing the problem, it is easiest and most economical to replace one component at a time in the following order:

(1) dispensing or aspirate tubing – that is, the input and output tubing (Figure 5–30), (2) syringe (Figure 5–30), and (3) 3-port valve (Figure 5–30).

If the plunger is stuck

Improper washing of a syringe may cause the plunger to get stuck. The following may help:

1. Remove the syringe and soak it in alcohol or detergent solution
2. If the plunger does not move after this, youneed to replace it.
3. If the plunger moves, rinse the syringe carefully with distilled or deionized water, remove the plunger, rinse it and allow the syringe and the plunger to dry separately.

Caution If the dispenser(s) is not properly installed, leakage may occur. ▲

Replacing the aspirate tube assembly or the complete dispensing tube assembly

To remove either the aspirate tube assembly, that is, the input tubing (Figure 5–30) or the complete dispensing tube assembly, that is, the output tubing (Figure 5–30), follow these steps:

1. To remove either the dispensing tube or the aspirate tube assembly from the valve, gently loosen the fittings manually. Unscrew the fittings and remove the tubing.
2. It is recommended to replace the complete dispensing tube assembly always when replacement is necessary.
3. To fit a new tubing, insert the fitting into the valve and tighten it finger tight.

Replacing a dispenser syringe

To replace a dispenser syringe (Figure 3–16, Figure 3–17 and Figure 5–31), follow these steps:

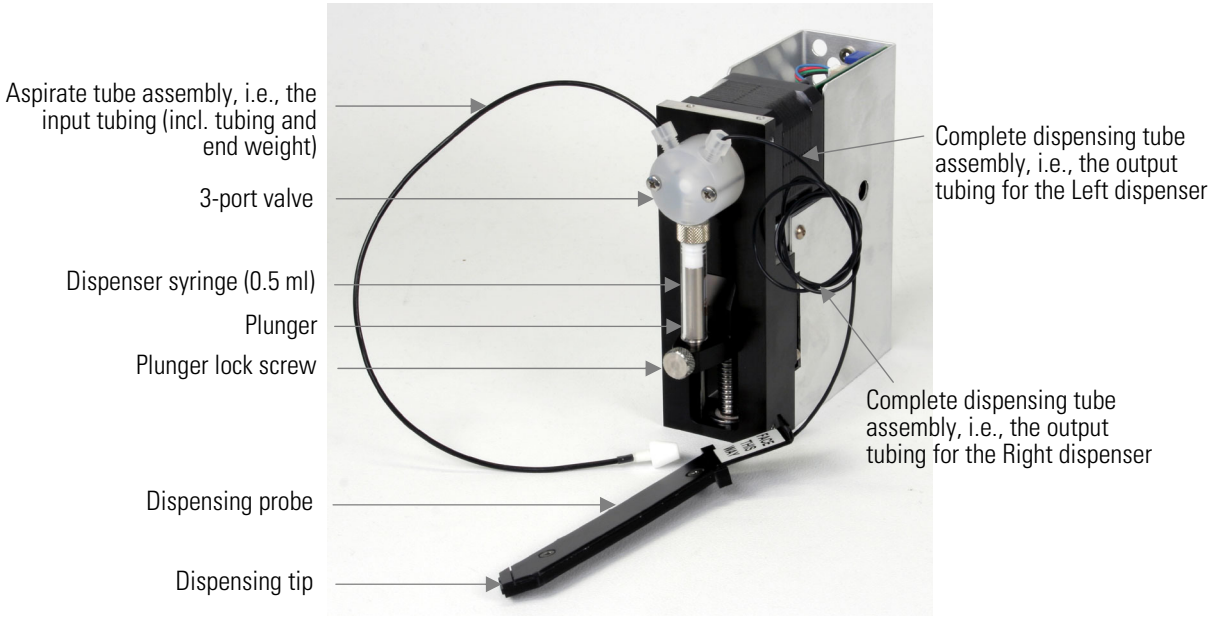


Figure 5–30. Dispenser assembly (unattached)

1. Remove the liquid from the dispenser syringe (Figure 5–30) and from the tubing.

2. Switch OFF the instrument (Figure 3–19 A) and then the mains power supply box (Figure 3–19 B) and disconnect the mains power supply cable.

3. Loosen the plunger lock screw (Figure 5–30) approximately three full turns clockwise (Figure 5–31, item c).

4. Pull the plunger holder arm (Figure 5–30) firmly down (Figure 5–31, item b).

5. Unscrew the syringe from the valve (Figure 5–31, item a).

6. To fit the new dispenser syringe, screw the syringe into the valve, pull the syringe plunger down to the plunger holder arm, and screw the syringe into place. Make sure the plunger lock screw is securely tightened (Figure 5–31). Also a 1.0 ml syringe is available on request.

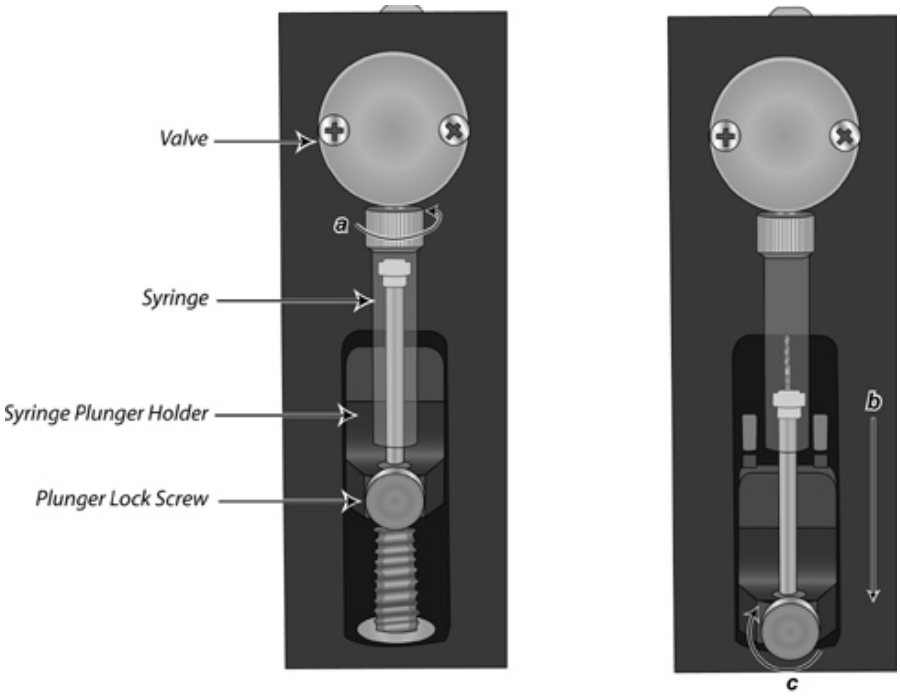


Figure 5–31. Replacing the dispenser syringe

Replacing the 3-port valve

To replace the 3-port valve (Figure 5–30 and Figure 5–32), it is recommended that you contact authorized technical service:

1. Remove the fluid from the dispenser(s).
2. Remove the syringe and tubing.
3. Remove the two Phillips head screws on the front of the valve, then remove the valve from the dispenser(s).
4. Install the new valve by placing it on the front panel so the screw holes line up. The valve coupler fitting mates to the valve motor shaft. The valve should be oriented with the tube fittings on top and the syringe on the bottom. Replace the valve screws.
5. Install the syringe and pull the syringe piunger until it is above the carriage.
6. Align the valve using the plunger as a guide and tighten from 1/8 to 1/4 turn after the syringe touch-off.
7. Pull the syringe plunger all the way into the carriage and secure by tightening the plunger lock screw.

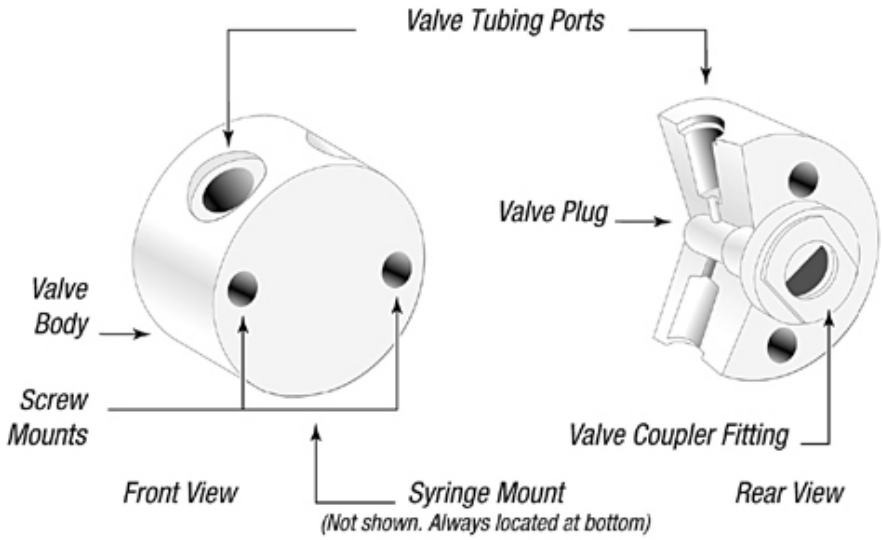


Figure 5–32. 3-port valve replacement

Replacing the filters in the filter slides

Introduction

This section describes the filters and filter slides used with the Appliskan as well as the replacement procedure.

A general outline of the filters and filter slides used is presented below.

Caution The filters must be the same in SkanIt Software for Appliskan as in the respective filter slide(s). The filter to be used is identified in the measurement protocol by its position in the filter slide. At the beginning of the measurement session, the filter information and position are checked. Measurement is not possible if the located slide code does not match the one specified in the protocol. ▲

Note Excitation and emission filters must not overlap! ▲

Excitation filters transmit energy within the absorption spectrum of the reagent. They also block other wavelengths, especially those important within the emission spectrum.

Emission filters transmit energy within the emission spectrum of the reagent. They block stray light from the sample and wavelengths within the excitation spectrum.

Typically each reagent has its own optimum excitation and emission filters. However, in some cases the wavelengths are such that two reagents can use the same filter(s).

In each of the fluorescence modes, as well as in absorbance detection, Appliskan employs optical filters.

In absorbance detection only one type of filter is needed. *Absorbance filters* are identical in size and use the same kind of filter slide as the excitation filters used in fluorescence detection.

In fluorescence polarization (FP), an excitation filter and two polarization emission filters are used. The emission filters allow readings at polarizations 90° apart.

If no filter is selected in your session in standard luminometric mode, one position of the filter slide must be empty. This does not apply to high-sensitive luminometric mode due to no filters being used in the measurement.

Procedure

SkanIt Software for Appliskan controls the filter change. Select Settings > Filters… > **Filter Slide** in SkanIt Software for Appliskan. Refer to the *SkanIt Software for Appliskan User Manual* (Cat. no. N05855).

Note The wavelength and bandwidth (HBW) of the filter are marked on the sticker on the plastic bag in which the filter is delivered. ▲

Caution Never touch the surfaces of the excitation/absorbance or emission filters. ▲

Caution Store the filters protected from dirt and dust. ▲

	Filter cover + screws
	Locking ring
	Filter with the arrow in the direction of the light path (the **arrow up** on the black filter rim)
	Filter slide

Figure 5–33. Inserting the filter into the filter slide

To install a filter into a four-position filter slide (Figure 5–33), follow these steps:

1. Select an empty position in the filter slide for the filter.

2. If the filter cover is attached, remove the screws attaching it to the filter slide with the crosshead screwdriver (excitation filter slide) or the hexagonal screwdriver (emission filter slide). Remove the filter cover.

3. Remove the metal locking ring with the filter assembling tool supplied (Figure 5–35). Two separate filter assembling tools are supplied, one slightly smaller for removing/inserting excitation/absorbance filters and one larger for emission filters (Figure 5–34). The filter assembling tool has two dents that are pushed into the dents of the metal locking ring.

Figure 5–34. Filter assembling tools for changing excitation and emission filters

Screw counterclockwise to remove a filter and clockwise to replace a filter (Figure 5–35). You open or close according to the direction of the thread.

Figure 5–35. Removing or replacing an emission filter

4. Insert the filter in the direction of the light path with the **arrow up** on the black filter rim or, if there is no arrow, insert the filter with the more reflective side down (Figure 5–36).

Note If a UV blocker is delivered (Figure 5–37), for example, with a TRF filter, insert it first before the filter. ▲

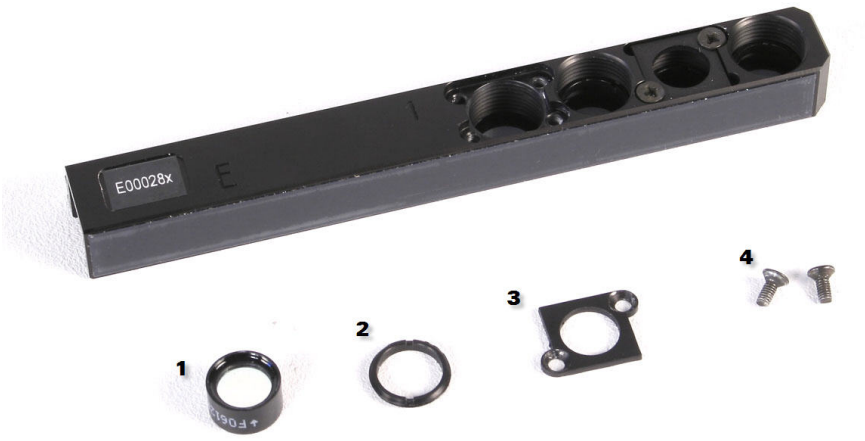
5. Insert the locking ring with the two dents facing up. Fasten the locking ring so that the filter remains in place.

Caution Do not screw the filter at a slant. The filter must be straight. Otherwise the light will diffract in a different way.

Also if there is surface tension left, the filter can break. ▲

6. Insert the filter cover. If you carry out FP measurements, refer to installation of polarizer films in “Installing a polarizer set” on page 72.

7. Replace and tighten the screws.



1. Filter in the direction of the light path
2. Locking ring
3. Filter cover
4. Filter cover screws

Figure 5–36. Parts of an excitation/absorbance filter slide

1. UV blocker, supplied only with some TRF filters
2. Filter
3. Locking ring
4. Filter cover
5. Filter cover screws

Figure 5–37. Parts of an emission filter slide

Installing a polarizer set

A polarizer set contains three polarizer films: one small film for the excitation filter and two large films for the emission filters. The small polarizer film is installed above the excitation filter in the excitation filter slide. The two large polarizer films are installed above the emission filters in the emission filter slide.

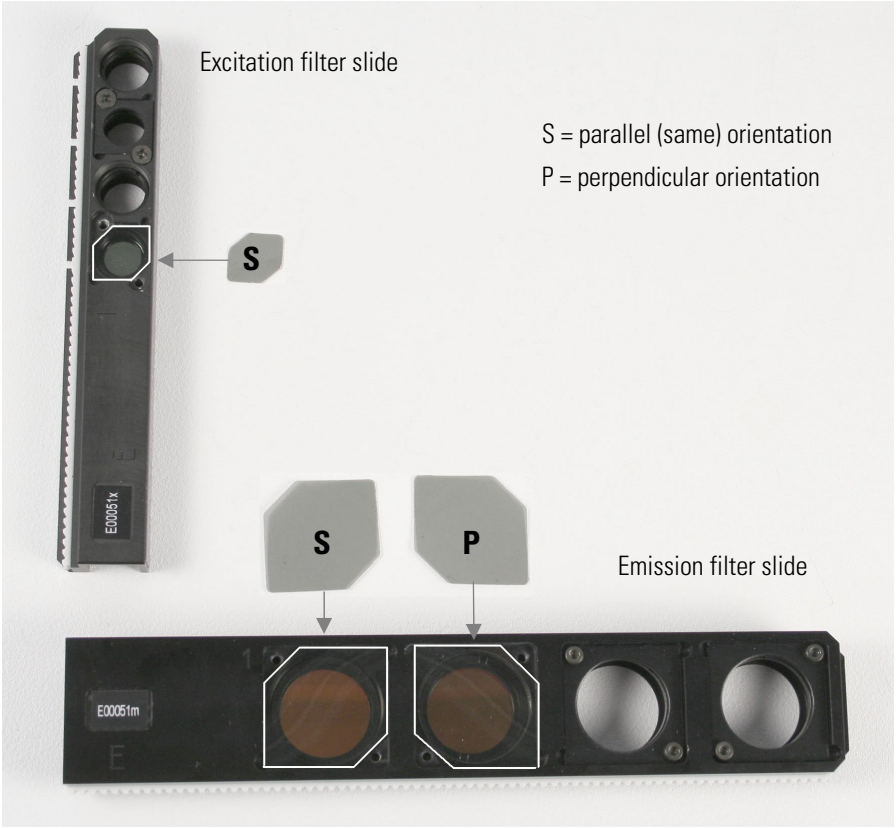


Figure 5–38. Parallel (S) and perpendicular (P) orientations of one small and two large polarizer films

When installing the polarizer films into the excitation and emission slide, keep the slides at a 90° angle in relation to each other (Figure 5–38). This helps you in inserting the polarizer films in the correct orientation. The orientations of the polarizer films are determined by the direction in which the slides are inserted into the instrument.

Before installing the polarizer set, one excitation filter and two emission filters, both with the same wavelength, should already be installed into the correct positions in the slides.

Items you need:

- Excitation filter slide
- Emission filter slide
- Excitation filter (already installed)
- Two emission filters with the same wavelength (already installed)
- Polarizer set (Cat. no. 425APP5990)
- Crosshead and hexagonal screwdrivers

Installing the small polarizer film to an excitation filter slide

To do before installing:

1. Select the filter position in the filter slide for the polarization film.

Note You can install polarizer films only into positions 1 and 3 in the excitation filter slide. ▲

2. If the filter cover is attached, remove the screws attaching it to the filter slide with the crosshead screwdriver. Remove the filter cover.

To install the polarizer film:

1. Peel off the protective films covering the polarizer film.

2. Insert the polarizer film with its convex side up in the S direction above the excitation filter as shown in Figure 5–39.

3. Insert the filter cover so that the screw holes do not overlap with the polarizer film.

4. Replace and tighten the screws.

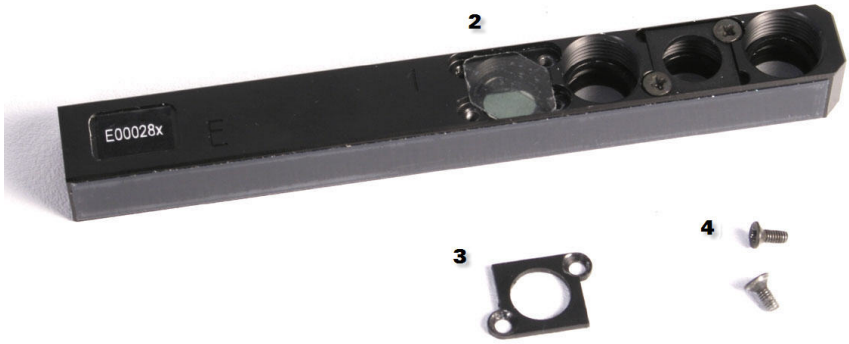


Figure 5–39. Installing the small polarizer film into an excitation filter slide

Installing the large polarizer films to an emission filter slide

To do before installing:

1. Select the two filter positions in the filter slide for the polarization films.

Note You can install polarizer films into any of the four positions in the emission filter slide. However, the two filters must be in adjacent positions. ▲

Note The two emission filters must have the same wavelength. ▲

2. If the filter covers are attached, remove the screws attaching them to the filter slide with the hexagonal screwdriver. Remove the filter covers.

To install the polarizer films:

1. Peel off the protective films covering the polarizer films.

2. Insert the first polarizer film with its convex side up in the S direction, which is the same direction in which the excitation polarizer is, above the first emission filter as shown in Figure 5–40.

3. Insert the second polarizer film with its convex side up in the P direction above the second emission filter as shown in Figure 5–40.

4. Insert the filter covers so that the screw holes do not overlap with the polarizer films.

5. Replace and tighten the screws.

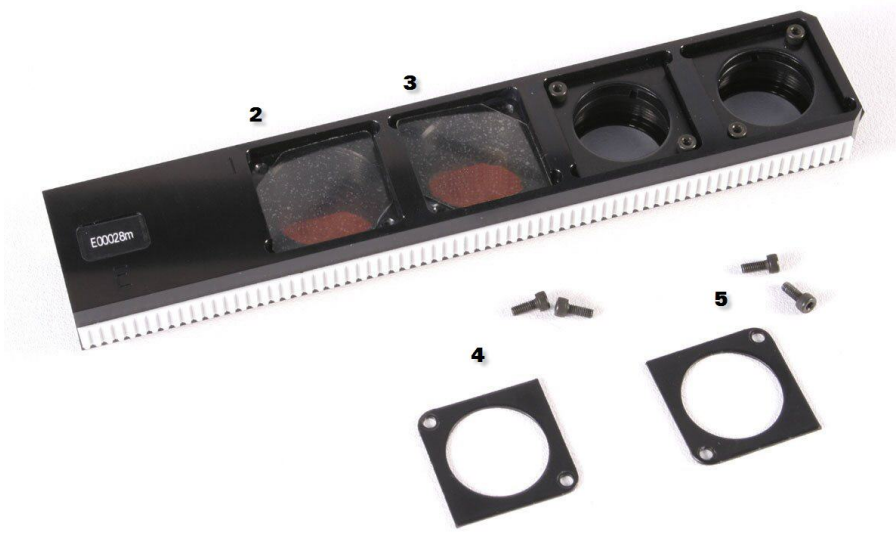


Figure 5–40. Installing the two large polarizer films into an emission filter slide

Disposal of materials

Follow laboratory and country-specific procedures for biohazardous or radioactive waste disposal. Refer to local regulations for the disposal of infectious material.

Warning The samples can be potentially infectious. Dispose of all used plates, strips, disposable gloves, syringes, disposable tips, and so on as biohazardous waste. ▲

Decontamination procedure

Decontamination should be performed in accordance with normal laboratory procedures. Any decontamination instructions provided with the reagents used should be followed.

A decontamination procedure is only recommendable when infectious substances have been in direct contact with any part(s) of the instrument.

If there is a risk of contamination with biohazardous material, the procedure recommended below or some other corresponding decontamination procedure must be performed.

It is strongly recommended to perform the complete decontamination procedure before relocating the instrument from one laboratory to another.

Decontamination is not required for the proper functioning of the instrument.

Example of decontaminants:

- Ethanol 70%
- Virkon solution 1 – 3%

- Glutaraldehyde solution 4%
- Chloramine T
- Microcide SQ 1:64

Caution If local or laboratory regulations prescribe regular decontamination, it is not advisable to use formaldehyde, since even small traces of formaldehyde negatively affect the enzyme being used in EIA tests resulting in bad test results. ▲

Warning The decontamination procedure should be performed by authorized trained personnel in a well-ventilated room wearing disposable gloves, protective glasses and clothing. ▲

1. Prepare the decontaminant: for example, 200 ml 4% glutaraldehyde solution (or another agent recommended by your safety officer).

2. Empty the plate carrier. Ensure that you are wearing disposable gloves.

3. Switch OFF the instrument (Figure 3–19 A) and then the mains power supply box (Figure 3–19 B) and disconnect the mains power supply cable.

4. Disinfect the outside of the instrument using a cloth dampened with 70% ethanol.

5. Place the instrument in a large plastic bag. Ensure that the dispenser cover and the emission and excitation/absorbance filter slide housing doors are open as well as the measurement chamber lid (Figure 2–5 and Figure 4–20).

6. Place a cloth soaked in the prepared solution into the bag. Ensure that the cloth does not come into contact with the instrument.

7. Close the bag firmly and leave the instrument in the bag for at least 24 hours.

8. Remove the instrument from the bag.

9. Clean the instrument using a mild detergent.

10. Remove any stains using 70% ethanol.

11. After performing this decontamination procedure, enclose a signed and dated Certificate of Decontamination (see Appendix B: "Certificate of Decontamination") both inside the transport package and attached to the outside of the package.

Maintaining a system log

A system log, which includes a short summary of the use, maintenance procedures, error messages and other information about the use of the system can be very useful in properly maintaining the system. The information in the log can frequently provide the service engineer with information that can assist in the diagnosis of problems and minimize the down time. An example of a typical user log is presented in Table 5–5.

The format of the log can vary to meet the overall requirements of the facility but should include all activity, problems, abnormal response and any other information that is relevant to the operation of the system.

Table 5–5. Example of a system log

User	Date	Comments
J. Smith	25/1/08	Emission filter changed in Em filter slide C. OK
C. Mayo	30/3/08	Complete tubing assembly replaced. OK
J. Smith	16/5/08	Annual service OK

A blank system log table that can be copied for use is in Appendix A: "System Log". Copy the table as many times as necessary, but leave the blank original inside the user manual.

How to pack for service

To pack for service, follow the instructions presented below.

Caution It is important that the instrument is thoroughly decontaminated before it is removed from the laboratory or any servicing is performed on it. ▲

When you ship the instrument for service, remember to:

- Inform about the use of hazardous materials.
- Decontaminate the instrument beforehand. Empty the dispenser(s) and remove any loose items from the plate carrier, for example, plates and the plate adapter before decontamination.
- Remove the complete dispensing tube assembly (Figure 5–30) after decontamination. Then replace the dispenser cover, the emission and excitation/absorbance filter slide housing doors and the measurement chamber lid.
- Pack the instrument according to the enclosed packing instructions.

- Use the original packaging to ensure that no damage will occur to the instrument during shipping. Any damage will incur additional labor charges.
- Enclose a dated and signed "Certificate of Decontamination" (see Appendix B: "Certificate of Decontamination") both inside and attached to the outside of the package, in which you return your instrument (or other items).
- Enclose the return authorization number (RGA) given by your local Thermo Fisher Scientific representative.
- Indicate the fault after you have been in touch with your local Thermo Fisher Scientific representative or the Thermo Fisher Scientific technical service department.

Refer to "General specifications" on page 81 for details on storage and transportation temperatures.

Service contracts

It is recommended to maintain and service the instrument regularly every 12 months on a contract basis by the manufacturer's trained service engineers. This will ensure that the product is properly maintained and gives trouble-free service. Contact the Thermo Fisher Scientific technical service department for more details.

Disposal of the instrument

If the Appliskan is exposed to potentially infectious chemical samples, toxic or corrosive chemicals or radioactive chemicals, waste management of the complete instrument must be carried out to ensure that there is no risk of contamination.

Warning Decontaminate the instrument before disposal. Refer to "Decontamination procedure" on page 75 and "Certificate of Decontamination" on page 97 about decontamination. ▲

Follow laboratory and country-specific procedures for biohazardous or radioactive waste disposal.

Dispose of the instrument according to the legislation stipulated by the local authorities concerning take-back of electronic equipment and waste. The proposals for the procedures vary by country.

Pollution degree	2 (see "Safety specifications" on page 84)
Method of disposal	Electronic waste Contaminated waste (Infectious waste)

WEEE symbol Thermo Fisher Scientific has contracted with one or more recycling/disposal companies in each EU Member State European Country, and this product should be disposed of or recycled through them. Further information on Thermo Fisher Scientific's compliance

with these Directives, the recyclers in your country, and information on Thermo Scientific products which may assist the detection of substances subject to the RoHS Directive are available at **www.thermo.com/WEEERoHS**.

Regarding the original packaging and packing materials, use the recycling operators known to you.

For more information, contact your local Thermo Fisher Scientific representative.

Chapter 6
Technical Specifications

General specifications

Thermo Fisher Scientific reserves the right to change any specifications without prior notice as part of our continuous product development program.

Table 6–6. Technical specifications

Technical specifications	
Overall dimensions	ca. 375 mm (W) x 495 mm (D) x 340 mm (H) [14.8" (W) x 19.5" (D) x 13.4" (H)]
Weight	Instrument: 27 kg [60 lbs.]; each dispenser adds 1.5 kg [3 lbs.] to the weight Mains power supply box: 3.6 kg [8 lbs.]
Operating conditions	+10°C to +40°C; maximum relative humidity 80% for temperatures up to 31°C decreasing linearly to 50% relative humidity at 40°C Indoor use only
Transportation conditions	-40°C to +70°C, packed in transport packaging
Storage conditions	-25°C to +50°C, packed in transport packaging
Mains power supply	100 – 240 Vac, 50/60 Hz, nominal
Power consumption	350 W max.
Heat dissipation	1195 BTU max.
User interface	The instrument is under PC control and run on SkanIt Software for Appliskan, which controls all the instrument functions and provides data reduction as well as reporting functions.
Computer interface	RS-232C
Light source	Xenon flash lamp
Detector	Photomultiplier tube for fluorometry (PMT1) and luminometry (PMT1 and PMT2) and a photodiode for photometry
Measurement types	Fluorescence intensity, time-resolved fluorescence, fluorescence polarization, absorbance and luminescence
Wavelength selection	By filters: Ex/Abs ∅ 12.5 mm, Em ∅ max. 25.4 mm
Plate types	6 to 384-well plates
Incubator	Measurement chamber
Shaker	Linear shaking
Dispenser(s)	Up to two optional dispensers

Performance specifications

This section provides the performance specifications for the relevant measurement techniques and other instrument capabilities.

Table 6–7. Fluorometry

Performance specifications / Fluorometry	
Wavelength selection	By filters
Excitation wavelength range	200 – 1000 nm
Emission wavelength range	360 – 820 nm
Xenon flash lamp	Lamp lifetime typically 10^9 flashes (10^6 96-well microplates using a 100 ms integration time per well)
Sensitivity	Fluorescence intensity: < 2 fmol fluorescein/well, 384-well plate Time-resolved fluorescence: < 20 amol Europium/well, 384-well plate
Precision	Fluorescence polarization: < 10 mP 1 nM fluorescein, 96-well plate
Dynamic range	> 5 decades
Measurement time	Fluorescence intensity: 10 – 1000 ms Time-resolved fluorescence: 5 – 10000 ms Fluorescence polarization: 10 – 10000 ms

Table 6–8. Photometry

Performance specifications / Photometry	
Wavelength selection	By filters
Wavelength range	200 – 1000 nm
Linearity	0 – 2.5 Abs (96-well plates) at 450 nm, ± 2% 0 – 2 Abs (384-well plates) at 450 nm, ± 2%
Measurement range	0 – 4 Abs
Absorbance resolution	0.001 Abs
Measurement time	20 – 1000 ms

Table 6–9. Luminometry

Performance specifications / Luminometry	
Wavelength selection	Standard mode: by filters High-sensitive mode: no filters
Emission wavelength range	Standard mode: 360 – 820 nm High-sensitive mode: 300 – 630 nm
Sensitivity	High-sensitive mode: < 10 amol ATP/well using flash reaction, white 384-well plate Standard mode: < 200 amol ATP/well using flash reaction, white 384-well plate
Dynamic range	> 5 decades
Measurement time	10 – 10000 ms

Table 6–10. Incubator

Performance specifications / Incubator	
Incubator warm-up time	From 25°C to 37°C, 30 min
Temperature range	From ambient + 4°C to 45°C at ambient 25°C
Setting range	From 20°C to 45°C in 0.1°C increments
Mean temperature of the wells	± 0.5°C at 37°C, ambient 25°C, covered 96-well plate
Temperature standard deviation	1°C at 37°C, ambient 25°C, covered 96-well plate
Liquid warm-up time	1 h from 25°C to 37°C, covered 96-well plate, 200 µl water/well

Table 6–11. Shaker

Performance specifications / Shaker	
Shaking method	Linear shaking
Shaking amplitude	1 – 10 mm

Table 6–12. Dispenser(s)

Performance specifications / Dispenser(s)	
Syringe size	500 µl (1000 µl on request)
Dispensing volume	5 – 500 µl with 1 µl increments
Dispensing accuracy	< 0.2 µl or 2%, whichever is greater, 5 – 500 µl
Dispensing precision	5 – 20 µl < 5%
Dead volume	800 µl, 100 µl with backflush (possibility to empty reagent back into the original reagent vessel)

Safety specifications

This section describes the safety specifications for the Appliskan instrument.

In conformity with the requirements

The Appliskan bears the following markings:
Type 2001
100 – 240 Vac, 50/60 Hz, 200 VA
CE mark

The Appliskan conforms to the following requirements:
2006/95/EC (Low Voltage Directive)
2004/108/EC (Electromagnetic Compatibility Directive, EMC)
FCC Part 15, Subpart B/Class B (July 2004)
2002/96/EC (Waste of Electrical and Electronic Equipment)

Safety performance:
EN 61010-1:2001 (Ed. 2) taking into account US and CA National differences

The safety specifications are also met under the following environmental conditions in addition to or in excess of those stated in the operating conditions:	
Altitude	Up to 2000 m
Temperature	+5°C to +40°C
Humidity	Maximum relative humidity 80% for temperatures up to 31°C decreasing linearly to 50% relative humidity at 40°C
Mains supply fluctuations	± 10% from nominal

EMC performance:	
EN 61000-6-3:2001	Generic standards – Emission standard for residential, commercial and light-industrial environments
EN 61000-6-1:2001	Generic standards – Immunity standard for residential, commercial and light-industrial environments
EN 61326-1:1997 + A1:1998 + A2:2001 + A3:2003	Product family standard

Test standards	Performance limits
EN 55022:1998 + A1:2000 + A2:2003	Class B, 150 kHz – 1 GHz
EN 61000-3-2:2000	Class A
EN 61000-3-3:1995 + A1:2001	
ANSI C63.4:2000	Class B, 150 kHz – 30 MHz; 30 MHz – 1 GHz
EN 61000-4-2:1995 + A1:1998 + A2:2001	4 kV CD, 8 kV AD, Criteria B
EN 61000-4-3:2002 + A1:2002	3 V/m, 80 MHz – 2 GHz, Criteria A
EN 61000-4-4:2004	1 kV, Criteria B
EN 61000-4-5:1995 + A1:2001	2 kV line to ground, 1 kV line to line, Criteria B
EN 61000-4-6:1996 + A1:2001	3 V_{rms}, 150 kHz – 80 MHz, Criteria A
EN 61000-4-8:1993 + A1:2001	3 A/m, Criteria A
EN 61000-4-11:1994 + A1:2001	30%/10 ms, Criteria B 60%/100 ms, Criteria B 100%/10 ms, Criteria B 100%/5 s, Criteria C

Chapter 7
Frequently Asked Questions

Q&As

Q1: What plate colors can be used for fluorescence intensity?

A1: Best performance is generally obtained with black plates, which also have the lowest background fluorescence. However, with some fluorochromes, white plates can also be used for obtaining a slightly better sensitivity.

Transparent or white plates can be used, but the sensitivity is often lower and the difference is dependent on the wavelengths used.

Q2: When can I use white plates in fluorometry?

A2: In certain assays, such as DNA quantification with PicoGreen, white plates can be used as the obtained fluorescent signal level is not very high. White plates can also be used in, for example, GFP quantification; however, white plates should not be used when the signal level is very high.

Q3: What plates can be used with the Appliskan?

A3: 6 to 384-well plates can be used with the Appliskan.

Table 7–13. Plates used with the Appliskan

Detection technology	Plates recommended
Fluorescence intensity (FI)	Solid black plates
Time-resolved fluorometry (TRP)	Solid white plates OR occasionally solid black plates (TR-FRET assays)
Fluorescence polarization (FP)	Solid black plates
Absorbance (Abs)	Solid clear, flat-bottom plates OR clear, flat-bottom plates with white or black walls
Luminescence	Solid white plates

Q4: What kind of adapters are available for the Appliskan?

A4: The adapter for SBS standard 96 to 384-well plates (Figure 2–13) is available for the Appliskan.

Q5: How do I calculate the concentration of samples from photometric readings in a microplate?

A5: Establish a standard curve on the plate and determine the concentration of unknowns based on the standard curve.

Q6: Can plates be used to directly measure the concentration of DNA or proteins?

A6: Yes, with plates suitable for UV measurements down to 260 nm.

Q7: How long does it take for the Appliskan incubator to reach 37°C?

A7: Approximately 30 minutes (from 25°C). However, warming up solutions takes about 1 hour.

Q8: What is the useful life of the lamp?

A8: Typically 10^9 flashes of reading of 1 million microplates (96 wells) by using the 100 ms measurement time.

Q9: What kind of lamp is used?

A9: A xenon flash lamp.

Q10: Can I use the Appliskan to measure the expression levels of reporter genes?

A10: Yes, Appliskan can be used to measure the expression level of any reporter gene that has either a photometric, fluorometric or a luminometric detection system available. Expression levels can be measured both from intact cells (for example, green fluorescent proteins and their variants) and from cell lysates (for example, photometric/fluorometric beta-galactosidase, alkaline phosphatase and CAT assays).

Q11: How often do I have to run a photometric verification plate?

A11: It is not necessary to read a photometric verification plate to ensure that the instrument is functioning properly. Startup checks for correct instrument operation are performed each time it is turned on. However, a verification plate is an external second source that documents instrument functionality. The frequency with which a verification plate is used depends on the standard operating procedures of each individual user.

Q12: What is the minimum time between measurements in kinetic measurements?

A12: 1 s in one well.

Q13: What is the maximum height of microplates used with the Appliskan?

A13: The maximum height of a plate with or without a lid is 20.5 mm without the adapter.

Q14: Does dispensing and measurement occur immediately after each other?

A14: No, there is a slight < 1 s delay between dispensing and measurement, since the dispensing position is not in the measurement position.

Chapter 8
Troubleshooting Guide

Note Do not use the instrument if it appears that it does not function properly. ▲

Note that the instrument does not verify the logic flow of the received commands.

Error messages

When an error is detected, the current operation is terminated. After an error, it is best to abort the current run and restart from the beginning after the problem is fixed. The error messages (Table 8–14) that may appear in SkanIt Software for Appliskan are presented below.

Table 8–14. Error messages reported

Error message	Suggested action
Error","Malfunction in excitation filter mechanism"	Run the excitation filter slide out and in. Contact service if the error recurs.
Error","Absorbance less than in air, probable malfunction"	Contact service.
Error","Malfunction in emission filter mechanism"	Run the emission filter slide out and in. Contact service if the error recurs.
Error","Overflow, either excitation filter missing or bad cable or electronics fault in the photodiode integrator"	Check the excitation filter. Contact service if the error recurs.
Error","Absorbance less than in air, may be malfunction"	Contact service if the error recurs.
Error","XP3000 Speed > limit, limit used"	Check the serial port. Contact service if the error recurs.
Error","XP3000 Speed < limit, limit used"	Check the serial port. Contact service if the error recurs.
Error", "electronic component failure, EEPROM chip on the CPU card"	Contact service.
Error", "Emission filter mechanism, (slide may be missing)"	Run the emission filter slide out and in. Contact service if the error recurs.
Error","LidSensor indicates close after open command"	Contact service if the error recurs.
Error","LidSensor indicates open after close command"	Contact service if the error recurs.
Error","Aperture Home sensor not found"	Contact service if the error recurs.

Continued

Cont.

Error message	Suggested action
Error","Aperture motor has lost steps"	Contact service if the error recurs.
Error", "Measuring position unclear, check protocol"	Contact service if the error recurs.
Error","Excitation filter position is not in range."	Check the serial port. Contact service if the error recurs.
Error","Unexpected protocol array, suggest defective CPU"	Contact service.
Error","Unexpected protocol array, indicates defective CPU"	Contact service.
Error","Filtered light intensity has changed since last filter calibration"	Contact service if the error recurs.
Error","Fluoro signal unrealistic, overflow because filter(s) missing or electronics fault"	Run the emission filter slide out and in. Contact service if the error recurs.
Error","Protocol not valid"	Check the serial port. Contact service if the error recurs.
Parameter error","Parameter conflict, Raster mode requires n*n type repeat (4,9,16,25..1024"	Check the serial port. Contact service if the error recurs.
Conveyor Error","Plate module"	Contact service if the error recurs.
Conveyor Error","Home invalid"	Contact service if the error recurs.
Conveyor","Conveyor cards do not respond, check multifunction cable CPU connector"	Contact service if the error recurs.
Conveyor Error","Conveyor Home and End sensor(s) active in the same time"	Contact service if the error recurs.
Conveyor Error","Plate carrier has lost its positional information, press stop to recalibrate"	Contact service if the error recurs.
Conveyor Error","Conveyor sensors are not calibrated"	Contact service if the error recurs.
Conveyor Error","Home sensor X ON state not detected"	Contact service if the error recurs.
Conveyor Error","Home sensor Y ON state not detected"	Contact service if the error recurs.
Conveyor Error","Home sensor Y OFF state not detected"	Contact service if the error recurs.
Conveyor Error","Home sensor X OFF state not detected"	Contact service if the error recurs.
Conveyor Error","Lost X steps > Current limit"	Contact service if the error recurs.
Conveyor Error","Lost X steps > Current limit"	Contact service if the error recurs.
Conveyor Error","Lost Y steps > Current limit"	Contact service if the error recurs.
Conveyor Error","Lost Y steps > Current limit"	Contact service if the error recurs.
Conveyor Error","Wrong attribute"	Contact service if the error recurs.
Conveyor Error","Unexpected end sensor_X ON state detected"	Contact service if the error recurs.
Conveyor Error","End sensor X ON state not detected"	Contact service if the error recurs.
Conveyor Error","End sensor Y ON state not detected"	Contact service if the error recurs.
Conveyor Error","End sensor Y OFF state not detected"	Contact service if the error recurs.
Conveyor Error","End sensor X OFF state not detected"	Contact service if the error recurs.
Conveyor Error","HomeSensor X position out of range 0.2..3 mm"	Contact service if the error recurs.
Conveyor Error","HomeSensor Y position out of range 0.2..3 mm"	Contact service if the error recurs.

Continued

Cont.

Error message	Suggested action
Conveyor Error","EndSensor X position out of range 0.2..3 mm"	Contact service if the error recurs.
Conveyor Error","EndSensor Y position out of range 0.2..3 mm"	Contact service if the error recurs.
Conveyor Error","Max steps between X sensors out of range"	Contact service if the error recurs.
Conveyor Error","Max steps between Y sensors out of range"	Contact service if the error recurs.
Conveyor Error","Repeatability in X movement out of range"	Contact service if the error recurs.
Conveyor Error","Repeatability in Y movement out of range"	Contact service if the error recurs.
Conveyor Error","Home invalid"	Contact service if the error recurs.
Conveyor Error","Move impossible X_target too small"	Contact service if the error recurs.
Conveyor Error","Move impossible Y_target too small"	Contact service if the error recurs.
Conveyor Error","Move impossible X_target too big"	Contact service if the error recurs.
Conveyor Error","Move impossible Y_target too big"	Contact service if the error recurs.
Conveyor", "Conveyor cards do not respond, check multifunction cable"	Contact service if the error recurs.
Conveyor Error","Next plate position mechanically impossible (Check plate definition, microstep, conveyor operation"	Contact service if the error recurs.
Label Error:","Unknown or unimplemented label type"	Check the serial port. Contact service if the error recurs.
Dispenser Error","No response from dispenser"	Contact service if the error recurs.
Dispenser Error","Incorrect response from dispenser"	Contact service if the error recurs.
Dispenser Error","No response from dispenser"	Contact service if the error recurs.
Dispenser Error","Initialization error"	Contact service if the error recurs.
Dispenser Error","Invalid command"	Contact service if the error recurs.
Dispenser Error","Invalid operand"	Contact service if the error recurs.
Dispenser Error","Invalid command sequence"	Contact service if the error recurs.
Dispenser Error","Fluid detection"	Contact service if the error recurs.
Dispenser Error","EEPROM Failure"	Contact service if the error recurs.
Dispenser Error","Dispenser is not initialized"	Contact service if the error recurs.
Dispenser Error","Unspecified error"	Contact service if the error recurs.
Dispenser Error","Plunger Overload"	Contact service if the error recurs.
Dispenser Error","ValveOverload"	Contact service if the error recurs.
Dispenser Error","Plunger move not allowed"	Contact service if the error recurs.
Dispenser Error","Unspecified error"	Contact service if the error recurs.
Dispenser Error","CommandOverflow"	Contact service if the error recurs.
Em. sensor","Emission sensor active in unexpected filter position(2..4)"	Run the filter slide out and in. Contact service if the error recurs.
Error Exc.slide","Excitation filter slide home position seek failed, indicates electronic fault"	Run the filter slide out and in. Contact service if the error recurs.
Error Exc.slide","Slide location failed"	Run the filter slide out and in.

Continued

Cont.

Error message	Suggested action
	Contact service if the error recurs.
Excitation light","Light intensity > Reference light intensity too low"	Check the lamp. Contact service if the error recurs.
Excitation light","Light intensity > Reference light intensity too high"	Check that there is a filter. Contact service if the error recurs.
Excitation light","Light intensity info lost"	Contact service if the error recurs.
Calib. failed","Light output over maximum, at minimum gain and light intensity"	Contact service if the error recurs.
Calib. failed","Light source failed, check:cables, ex.filter, slide_position, flashlamp, lamp power, photodetector"	Contact service if the error recurs.
Overflow","Emission is too strong, reduce gain (setting is in the protocol)"	Check that there is a filter. Contact service if the error recurs.
Overflow","Counts over 524287 (19 bit counter) in one flash, check HV and threshold"	Contact service.
Overfow","Unrealistic counts, check HV and threshold"	Contact service.
ADC failure", "ADC component failure or low voltage"	Contact service.
ADC failure","ADC chip in the CPU card does not respond correctly"	Contact service.
ADC failure","A/D converter in the CPU card is not responding correctly"	Contact service.
ADC error","ADC chip does not respond"	Contact service.
ADC error","Unexpected voltage at integratorA output"	Contact service.
ADC error","Unexpected voltage at fluoro integrator output"	Contact service.
ADC error","Unexpected voltage at reference integrator output"	Contact service.
ADC error","Unexpected voltage at absorbance integrator output"	Contact service.
ADC error","Unexpected voltage at offset"	Contact service.
PMT LightShutter","Light shutter home position is not detected"	Contact service if the error recurs.
PMT LightShutter","Light shutter position indicates open after close"	Contact service if the error recurs.
PMT LightShutter","Light shutter position indicates close after open"	Contact service if the error recurs.
Motor error","Stepper card (connected to motorized lid) does not respond, check card and multifunction cable"	Contact service.
Motor error","Stepper card (connected to absorbance light shutter) does not respond, check card and multifunction cable"	Contact service.
Motor error","X_motor card do not respond, check card(address) and multifunction cable"	Contact service.
Motor error","Y_motor card do not respond, check card(address) and multifunction cable"	Contact service.
Motor error","Z_motor card do not respond, check card and multifunction cable"	Contact service.
Motor error","W_motor card (connected to light shutter) does not respond, check card and multifunction cable"	Contact service.
DC motor error","G_motor (PMT pos) card does not respond, check card and	Contact service.

Continued

Cont.

Error message	Suggested action
multifunction cable"	
Plate zone error","FirstUsedWell < 1"	Check the serial port. Contact service if the error recurs.
Plate zone error","LastUsedWell < FirstUsedWell"	Check the serial port. Contact service if the error recurs.
Plate zone error","LastUsedWell > Wells"	Check the serial port. Contact service if the error recurs.
Plate zone error","FirstUsedRow < 1"	Check the serial port. Contact service if the error recurs.
Plate zone error","LastUsedRow < FirstUsedRow,PARAMETER_ERROR"	Check the serial port. Contact service if the error recurs.
Plate zone error","LastUsedRow > Rows in the plate"	Check the serial port. Contact service if the error recurs.
Plate zone error","FirstUsedColumn < 1"	Check the serial port. Contact service if the error recurs.
Plate zone error","LastUsedColumn < FirstUsedColumn"	Check the serial port. Contact service if the error recurs.
Plate zone error","LastUsedColumn > Columns in the plate"	Check the serial port. Contact service if the error recurs.
Plate zone error","FirstUsedWell < 1"	Check the serial port. Contact service if the error recurs.
Plate zone error","LastUsedColumn > Columns in the plate"	Check the serial port. Contact service if the error recurs.
Plate zone error","FirstUsedWell < 1"	Check the serial port. Contact service if the error recurs.
Heater error","Heater cards do not respond, check multifunction cable CPU connector"	Contact service.
Heater error","Top heater card does not respond"	Contact service.
Heater error","Under-side heater card does not respond"	Contact service.
Heater error","Heater power failed, check 24V power supply"	Contact service.
Heater error","Top heater power not available, check 24V power cabling"	Contact service.
Heater error","Under-side heater power not available, check 24V power cabling to conveyor unit"	Contact service.
SICO error","No voltage present in down PMT D1"	Contact service.
SICO error","No voltage present in down PMT D1"	Contact service.
SICO error","down PMT D1 voltage on when switched off"	Contact service.
SICO error","top PMT D1 voltage on when switched off"	Contact service.
SICO error","SICO (Analyser unit) is connected to wrong connector"	Contact service.
SICO error","SICO (Analyser unit) is not connected or cable failure"	Contact service.
SICO error","SICO (Analyser unit) is not powered, check power cable"	Contact service.

Continued

Cont.

Error message	Suggested action
Analyser fault", "photon counting comparator does not work"	Contact service.
PROM error","PROM checksum indicates faulty PROM"	Contact service.

If any other error messages occur, contact service.

Service request protocol

If the Appliskan requires service, contact your local Thermo Fisher Scientific representative or the Thermo Fisher Scientific technical service department. Do not under any circumstances send the instrument for service without any prior contact. It is imperative to indicate the fault and nature of the required service. This will ensure a faster return of the instrument to the customer.

Your local Thermo Fisher Scientific representative or distributor will take care of sending a complaint form (that is, the Warranty Claim Technical Sheet) to the Thermo Fisher Scientific technical service department. The Warranty Claim Technical Sheet contains a more detailed description of the fault, symptom or condition. Give all the necessary information to the distributor, who will fill out and forward the Warranty Claim Technical Sheet to the Thermo Fisher Scientific technical service department.

Check “How to pack for service” on page 77. You will find instructions on how to proceed before shipping the instrument for service to Thermo Fisher Scientific Oy.

Check that any necessary decontamination procedure has been carried out before packing. Refer to “Decontamination procedure” on page 75 and “Certificate of Decontamination” on page 97. Ensure that the Certificate of Decontamination (see Appendix B: “Certificate of Decontamination”) as well as the return authorization number (RGA) are sent with the instrument.

The Thermo Fisher Scientific technical service department will keep you up to date with the progress of service and provide you with any further details you might need, for example, on maintenance, serviceability, troubleshooting and replacement.

Certificate of Decontamination

The decontamination procedure is required before shipping the instrument to Thermo Fisher Scientific Oy, for example, for repair. If, for any reason, the instrument is shipped back to Thermo Fisher Scientific Oy, it must be accompanied by a dated and signed Certificate of Decontamination, which must be attached to the outside of the package containing the instrument. Refer to Appendix B: “Certificate of Decontamination” and “Decontamination procedure” on page 75.

Failure to confirm decontamination will incur additional labor charges or at worst the items will be returned for proper cleaning. Before returning any instrument(s) or item(s), ensure that they are fully decontaminated. Confirm A or B status. Refer to Appendix B: “Certificate of Decontamination”.

Chapter 9
Ordering Information

Appliskan

Contact your local Thermo Fisher Scientific representative for ordering and service information.

Table 9–15. Instrument catalog number

Code	Instrument / System
5230000	Appliskan, 100-240 V, 50/60 Hz
5230010	Appliskan with one dispenser, 100-240 V, 50/60 Hz
5230020	Appliskan with two dispensers, 100-240 V, 50/60 Hz

List of spare parts and accessories

Table 9–16. Codes for spare parts and accessories

Code	Item	Quantity
N05853	*Appliskan User Manual*	1
N05855	*SkanIt Software for Appliskan User Manual*	1
5187060	SkanIt Software for Appliskan, Research Edition	1
425SP9910	Power supply	1
460SP300	Adapter for SBS standard plates	1
310SP90605	Spare fuse 5A	1
425APP9201	Filter slide for 12.5 mm (0.5") Excitation and Abs, filters, code A	1
425APP9202	Filter slide for 12.5 mm (0.5") Excitation and Abs, filters, code B	1
425APP9203	Filter slide for 12.5 mm (0.5") Excitation and Abs, filters, code C	1
425APP9204	Filter slide for 12.5 mm (0.5") Excitation and Abs, filters, code D	1
425APP9200	Filter slide for 12.5 mm (0.5") Excitation and Abs, filters, code E	1
425APP9211	Filter slide for 25 mm (1") Emission filters, code A	1
425APP9212	Filter slide for 25 mm (1") Emission filters, code B	1
425APP9213	Filter slide for 25 mm (1") Emission filters, code C	1
425APP9214	Filter slide for 25 mm (1") Emission filters, code D	1
425APP9210	Filter slide for 25 mm (1") Emission filters, code E	1
431APP401	Excitation/absorbance filter assembling tool	1
431APP402	Emission filter assembling tool	1
460SP320	Dispensing tube assembly, 2 tubes (Left and Right)	1
460SP310	Dispensing tube assembly, 1 tube (Left)	1

Continued

Code	Item	Quantity
2805690	Aspirate tube assembly, incl. tubing and end weight	1
431SP420	Dispenser syringe 0.5 ml	1
SP-00100	3-port valve (1/4-28 fittings)	1
24071700	Bottle stand	1
529SP010	RS-232C serial cable D9 Female/D25 Female	1

Filters

Table 9–17. Codes for filters

Code	Item	Quantity
	Fluorometric excitation filters	
425APP2355	Excitation filter 355nm HBW40 D12.5mm	1
425APP2485	Excitation filter 485nm HBW10 D12.5mm	1
425APP2544	Absorbance/Excitation filter 544nm HBW20 12.5mm	1
425APP2313A	Excitation filter 313nm HBW10 D12.5mm	1
425APP2340A	Excitation filter 340nm HBW25 D12.5mm	1
425APP2390A	Excitation filter 390nm HBW10 D12.5mm	1
425APP2532A	Excitation filter 532nm HBW10 D12.5mm	1
425APP2577A	Excitation filter 577nm HBW10 D12.5mm	1
	Fluorometric emission filters	
425APP3460	Emission filter 460nm HBW20 D25mm	1
425APP3535	Emission filter 535nm HBW20 D25mm	1
425APP3590	Emission filter 590nm HBW20 D25mm	1
425APP3400A	Emission filter 400nm HBW70 D25mm	1
425APP3420A	Emission filter 420nm HBW10 D25mm	1
425APP3500A	Emission filter 500nm HBW40 D25mm	1
	TRF excitation filter	
425APP2340	Excitation filter 340nm HBW80 D12.5mm	1
	TRF emission filter	
425APP3616	Emission filter 616nm HBW8.5 D25mm	1
	Absorbance filters	
425APP4405	Absorption filter 405nm HBW10 D12.5mm	1
425APP4416	Absorption filter 416nm HBW10 D12.5mm	1
425APP4450	Absorption filter 450nm HBW10 D12.5mm	1
425APP4492	Absorption filter 492nm HBW10 D12.5mm	1
425APP2544	Absorption/Excitation filter 544nm HBW20 12.5mm	1
425APP4595	Absorption filter 595nm HBW10 D12.5mm	1

Continued

Cont.

Code	Item	Quantity
425APP4620	Absorption filter 620nm HBW10 D12.5mm	1
425APP4254	Absorption filter 260nm HBW10 D12.5 mm	1
425APP4280	Absorption filter 280nm HBW10 D12.5 mm	1
425APP4508A	Absorption filter 508nm HBW10 D12.5mm	1
425APP4550A	Absorption filter 550nm HBW10 D12.5mm	1
425APP4656A	Absorption filter 656nm HBW10 D12.5mm	1
425APP4750A	Absorption filter 750nm HBW10 D12.5mm	1
	Fluorescence polarizer set	
425APP5990	Polarizer set	1

For other filters, please contact your Thermo Fisher Scientific representative.

Chapter 10
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Literature

The section on literature is divided according to the relevant measurement techniques.

Fluorescence intensity

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Appendix A
System Log

Instrument name/number:

User	Date	Comments

PHOTOCOPIABLE

Appendix B
Certificate of Decontamination

Name: _____

Address: _____

Tel./Fax: _____

Name: _____ Serial no.: _____

A) I confirm that the returned items have not been contaminated by body fluids, toxic, carcinogenic or radioactive materials or any other hazardous materials.

B) I confirm that the returned items have been decontaminated and can be handled without exposing the personnel to health hazards.

Materials used in the unit: Chemicals + Biological • Radioactive *)

Specific information about contaminants:

_____

_____

Decontamination procedure[1]:

_____

_____

Date and place: _____

Signature: _____

Name (block capitals): _____

*) The signature of a Radiation Safety Officer is also required when the unit has been used with radioactive materials.

This unit is certified by the undersigned to be free of radioactive contamination.

Date and place: _____

Signature: _____

Name (block capitals): _____

PHOTOCOPIABLE

[1] Please include decontaminating solution used.

Appendix C
Thermo Scientific Appliskan Feedback Form

Instrument: Appliskan ☐	Instrument serial no.:
Software serial no. (from the Thermo Scientific SkanIt Software for Appliskan installation CD cover):	
PURCHASED BY	**PURCHASED FROM**
Company/Institute	Distributor
Department	Address
Address	
Tel.	Tel.
Fax	Date of delivery
Internet home page	
Date of purchase	
Your application area	
Dr. ☐ Mr. ☐ Mrs. ☐ Ms. ☐	Job title/Position
Surname (block capitals)	First name (block capitals)
Internet e-mail address	

	Excellent	Above expectations	As expected	Below expectations	Comments
Instrument installation	☐	☐	☐	☐	
Ease of use	☐	☐	☐	☐	
Flexibility	☐	☐	☐	☐	
User manual	☐	☐	☐	☐	
Software	☐	☐	☐	☐	
Customer support	☐	☐	☐	☐	
Overall	☐	☐	☐	☐	

Additional instrument and/or software features desired:

Did you encounter any problems?

Where did you first learn about the product?

Would you like to receive information about other Thermo Scientific products?

Glossary

absorbance (optical density) A logarithmic function of the transmission of a wavelength of light through a liquid. log (I/I_0) dimension [A]

adapter The elevation device used to hold and raise SBS standard plates in the plate carrier.

AFP Alpha-fetoprotein, common label in FRET and TR-FRET assays.

cAMP cyclic AMP (cyclic adenosine monophoshate). 3'5'-cyclic ester of AMP. The first second messenger hormone signalling system to be characterized. Generated from ATP by the action of adenyl cyclase that is coupled to hormone receptors by G-proteins (GTP-binding proteins). cAMP activates a specific (cAMP-dependent) protein kinase and is inactivated by phosphodiesterase action giving 5' AMP. Also functions as an extracellular morphogen for some slime moulds.

anisotrophic Describing a substance that exhibits different properties along different axes of propagation or for different polarizations of a traveling wave.

anisotrophy Term sometimes associated with the FP field. Polarization and anisotrophy are both derived from the measured vertical and horizontal intensities. The values are mathematically related and easily interconverted. Both values represent a weighted average of the bound versus unbound states of the fluorescent molecule. Anisotrophy is the preferred quantity because anisotrophy values are mathematically easier to manipulate in many FP studies. Cf. FA and FP.

aspirate/dispense tubing Connects the valve output port (1/4–28 thread) to a sample source and destination. The aspirate tubing is used to fill the syringe with reagent. The dispensing tube is used to dispense reagent from the syringe into a microplate.

ATP Adenosine triphosphate, a biological molecule that is commonly used as a reference chemical for luminometric sensitivity.

bioluminescence Naturally occurring chemiluminescence from light-emitting organisms, e.g., glowworms, some deep-sea fish, some bacteria and some fungi.

BRET Bioluminescence resonance energy transfer. Refer to "Luminescence" on page 22.

chemiluminescence Luminescence as a result of pure chemical reactions.

crosstalk Interfering signal from neighboring wells.

decade Order of magnitude. A logarithmic value that is used for presentation of dynamic range.

decontamination Removal or neutralization of radiologic, bacteriological, chemical or other contamination.

disinfection The destruction of pathogenic bacteria, usually with an antiseptic chemical or disinfectant.

dynamic range Refers to the range of signals an instrument can read, from the minimum to the maximum detectable. For example, dynamic range of seven decades means that the difference between the lowest and highest signals that can be measured is 10^7.

emission The release of light from a fluorochrome when an electron falls from an excited state to a lower energy state of the molecule.

error message Indication that an error has been detected.

excitation The absorption of light energy by a fluorochrome, during which electrons in the fluorochrome molecule are boosted to a higher energy level.

fluorescein An example of a fluorescent dye emitting green fluorescence.

fluorescence The emission of light from a fluorochrome, the wavelength of the light generally being of longer wavelength than that of the absorbed light.

fluorescence anisotrophy (FA) Generally the term FP is used instead of FA because FP is most often the term used to describe the entire technology. Cf. anisotrophy and FP.

fluorescence lifetime The period of time elapsed between when a fluorophore is excited and when it emits light. This is between 4 and 10 ns for most standard fluorophores and roughly 1 µs for long-lived lanthanides used for TRF measurements. Cf. τ (tau).

fluorescence polarization (FP) Homogeneous technique to study molecular interactions. FP predominates as the quantity in drug discovery. Cf. anisotrophy and FA.

fluorochrome (fluorophore) A molecule or chemical group that emits fluorescence.

fluorometer Instrument used for measuring the intensity of fluorescent radiation. Also known as fluorimeter.

fluorometry The measurement of fluorescence. Also known as fluorimetry.

FRET Fluorescence resonance energy transfer. Refer to “Fluorescence intensity” on page 20.

G factor Fluorescence anisotrophy measurements can be corrected for the varying efficiencies of each optical component: this correction is expressed as the G factor. The G factor can be obtained by measuring a sample with a known polarization value. G is typically between 0.8 and 1.2.

GPCR G-protein coupled receptor. Cell surface receptors that are coupled to heterotrimeric G proteins (GTP-binding proteins). All G-protein coupled receptors seem to have seven membrane-spanning domains (are serpentine receptors), and have been divided into two subclasses: those in which the binding site is in the extracellular domain, e.g., receptors for glycoprotein hormones, such as thyroid-stimulating hormone (TSH) and follicle-stimulating hormone (FSH); and those in which the ligand-binding site is likely to be in the plane of the seven transmembrane domains, e.g., rhodopsin and receptors for small neurotransmitters and hormones, e.g., muscarinic acetylcholine receptor.

initialization Initialization tests are so-called self-tests, which are carried out before operation to ascertain that the necessary instrument adjustments have been carried out.

LED Light-emitting diode.

luciferase A generic name for enzymes commonly used in nature for bioluminescence.

luminescence Emission of light (other than from thermal energy causes) such as bioluminescence.

luminometer An instrument used for measuring the intensity of luminescent radiation.

luminometric label (luminophore) A substance which emits light at room temperature. A group of atoms that can make a compound luminescent.

mP value The value that measures the fluorescence polarization. In assays the mP values are commonly between 50 and 500 mP (milliP).

multiplexing When two or more labels are used in the assay either simultaneously or consecutively, e.g., in fluorometric FRET, luminometric dual reporter gene and dual-label TRF assays.

optical density (absorbance) The amount of light passing through a sample to a detector relative to the total amount of light available. Optical density includes absorbance of the sample plus light scatter from turbidity. log (1/transmittance) = log (I/I_0) dimension [*O.D.*]

photometer A device measuring absorbance or optical density.

photometry The measurement of the properties of light, particularly (luminous) intensity.

photomultiplier tube (PMT) A photoelectric cell that converts light into electric current and amplifies the current.

polarization A measure of the extent of molecular rotation during the period between excitation and emission. The measured polarization is a weighted average of the two values, thus providing a direct measure of the fraction of tracer bound to receptor.

polarized light Linearly polarized light consists of only one oscillation direction.

polarizer An optical device capable of transforming unpolarized or natural light into polarized light, usually by selective transmission of polarized rays.

priming Completely filling the dispenser tubing and syringe with bubble-free fluid to allow a sustained, reproducible dispensing action. The air in an unprimed line acts as a spring, adversely affecting accuracy and precision.

quantum yield (Q) The ratio of the number of emitted photons to the number of excited molecules. Fluorophores differ in quantum yield, the higher the Q value, the more fluorescent the compound is. The theoretical maximum of Q=1 is for a highly fluorescent compound, and Q=0 corresponds to a non-fluorescent compound.

RFU or rfu Relative Fluorescence/Fluorometric Units. The arbitrary units in which fluorescence intensity is reported.

RH Relative humidity.

RLU or rlu Relative Luminescence/Luminometric /Light Units. The arbitrary units in which luminescence intensity is reported.

self-tests Initialization tests and adjustments that the instrument performs before operation as well as autocalibration.

Stokes shift The difference between the wavelengths of the excitation and emission peaks.

τ (tau) Fluorescence lifetime of the TRF label. Cf. fluorescence lifetime.

transmittance The ratio of transmitted (I) and incident light (I_0), I/I_0.

TRF Time-resolved fluorometry/fluorescence. Fluorescence intensity measurement using special labels.

TRF delay Waiting period between the excitation flash end and the beginning of the emission light measurement.

TR-FIA Time-resolved fluoroimmunoassay.

TR-FRET Time-resolved fluorescence resonance energy transfer.

Z factor (Z') Dimensionless, a simple statistical characteristic of a HTS assay. The Z factor is a characteristic parameter for the quality of the assay itself. For an assay to be very robust, it is necessary to have Z values greater than 0.5.

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Thermo Fisher Scientific Oy
Microplate Instrumentation
Ratastie 2, P.O. Box 100
FI-01621 Vantaa
Finland

www.thermo.com

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