Thermo SCIENTIFIC

Fluoroskan Ascent®

Fluoroskan Ascent® FL & **Fluoroskan** Ascent®

User Manual

User Manual Rev. 2.4; Nov. 2008, Cat. no. 1506450

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Symbols and Markings

SYMBOLS USED IN THE FLUOROSKAN ASCENT FL AND FLUOROSKAN ASCENT

Power ON

Power OFF

Connection to the protective grounding system

WARNING MARKINGS USED ON THE INSTRUMENT

Caution: risk of electric shock.

Caution: risk of personal injury to the operator or a safety hazard to the surrounding area. See the accompanying documentation.

CE compliance mark

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

WARNING MARKINGS USED IN THE DOCUMENTATION

Caution: risk of electric shock.

Caution: risk of personal injury to the operator or a safety hazard to the surrounding area.

Caution: risk of serious damage to the instrument, other equipment or loss of performance or function in a specific application.

Caution: biohazard risk.

Fluoroskan Ascent, Cat. no. 5210470 and 5210480 (with dispenser)
Fluoroskan Ascent FL, Cat. no. 5210450 and 5210460 (with dispenser)

User Manual Rev. 2.4; Nov. 2008, Cat. no. 1506450

Patents

The Fluoroskan Ascent FL and Fluoroskan Ascent have national and international patents and patents pending.

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1. Introduction

The Thermo Scientific Fluoroskan Ascent designed and made by Thermo Fisher Scientific is a microplate fluorometer which offers versatility and flexibility for even the most demanding fluorometric applications. The extended Thermo Scientific Fluoroskan Ascent FL is equipped with both fluorometric and luminometric detection technologies. As a combination instrument it covers the full range of fluorometric as well as glow and flash luminometric applications.

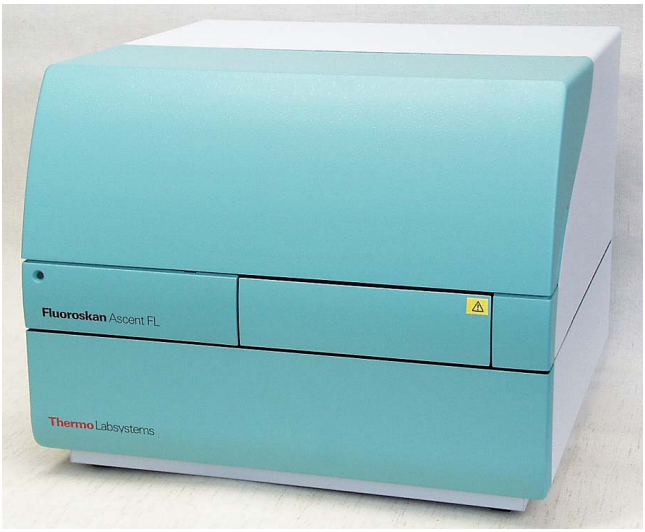


Fig. 1. Fluoroskan Ascent FL/Fluoroskan Ascent

Thermo Scientific Ascent Software controls all the reader functions and provides the features for data handling and report formatting. The software for the Fluoroskan Ascent is a dedicated software for fluorometric applications, while the software for the Fluoroskan Ascent FL enables both fluorometric and luminometric detection methods to be used, even during a single session.

The advanced optical system based on direct illumination optics, produces a sharply focused light beam for fluorometric measurements, prevents crosstalk and ensures accurate readings. The choice of two beam diameter settings allows optimal reading of 1 to 96 and 384 wells. High sensitivity is one of the main benefits of fiberless optics and a critical feature for luminometry.

The Fluoroskan Ascent FL can also be equipped with filters for luminometric applications.

Up to three reagent dispensers can be fitted on-board, making the reagent addition simple and highly accurate. The ability of the instrument to dispense and measure simultaneously enables the detection of flash luminescence reactions, fluorometric Ca^{2+} measurements and other rapid kinetic applications. For assays requiring temperature control, these instruments have an on-board incubator. Built-in orbital shaking speeds up reaction times and ensures effective mixing.

Robotic integration is simple and effective with the Fluoroskan Ascent FL and Fluoroskan Ascent. The plate carrier allows convenient access for the robotic arm and Ascent Software is easy to integrate with robotic and HIS/LIMS systems. The Fluoroskan Ascent FL and Fluoroskan Ascent are also fully compatible with Thermo Scientific robotic plate handling devices, expanding the measurement capacity. For further information, contact your local Thermo Fisher Scientific representative.

1.1 Intended use

1. The Fluoroskan Ascent is a high-quality microplate fluorometer intended for laboratory research use by professional personnel. The Fluoroskan Ascent is used to measure fluorescence from suitable 1- to 384-well plates mentioned in this manual. It also has incubation, shaking and reagent dispensing capabilities.
2. The Fluoroskan Ascent FL is a combined high-quality microplate fluorometer and luminometer intended for laboratory research use by professional personnel. The Fluoroskan Ascent FL is used to measure fluorescence or luminescence from suitable 1- to 384-well plates mentioned in this manual. It also has incubation, shaking and reagent dispensing capabilities.
3. For verification of the entire system, it is recommended that Good Laboratory Practices (GLP) be followed to guarantee reliable analyses.
4. Use for self-testing is excluded.

1.2 Method descriptions

1.2.1 Fluorometric measurement principle

Measurement with the Fluoroskan Ascent FL and Fluoroskan Ascent occurs in a narrow angle. The source of the excitation light and the detector of the emission light are both located on the same side of the microplate. Filters are held in 8-position filter wheels. The Fluoroskan Ascent FL/Fluoroskan Ascent can carry out both top and bottom reading. The position of the whole optic unit is easily changed.

1.2.2 Luminometric measurement principle (Fluoroskan Ascent FL)

A luminometric measurement uses the same optics as the fluorometric but the lamp is switched off. The emission filter slots 7 and 8 are reserved only for luminometric measurements. Filter slot 7 is empty for measurement and filter slot 8 is blocked to enable measurement of the PMT (photomultiplier tube) dark current. This feature is important to obtain optimal sensitivity. The light path from the measurement well to the first lens is protected by a light shield (Fig. 4.7a and Fig. 4.7b). The light shield is required with 96- and 384-well plates to avoid crosstalk in luminometric measurements. The light shield is not necessary, but does not interfere, in fluorometric measurements. When plates are higher than 15 mm, the light shield must be removed.

If the 384-well plate has a height of less than 15 mm, then an adapter must be used below the plate to raise the plate to the proper height. This adapter for low 384-well plates (Fig. 3.6:4) is included in the Fluoroskan Ascent FL instrument and can also be ordered separately.

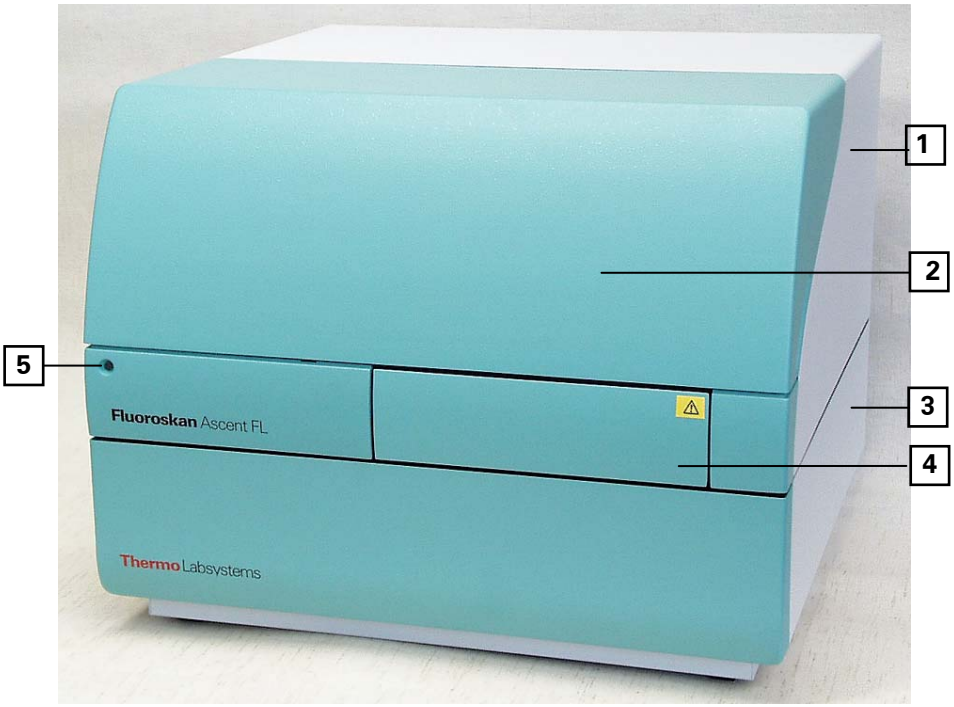
Caution: A luminometric measurement without a light shield may cause extra crosstalk.

Caution: The light shield must be removed with higher plates.

Note: The emission filter slots 7 and 8 are reserved only for luminometric measurements. Filter slot 7 is empty for measurement and filter slot 8 is blocked to enable measurement of the PMT (photomultiplier tube) dark current.

2. Instrument Layout

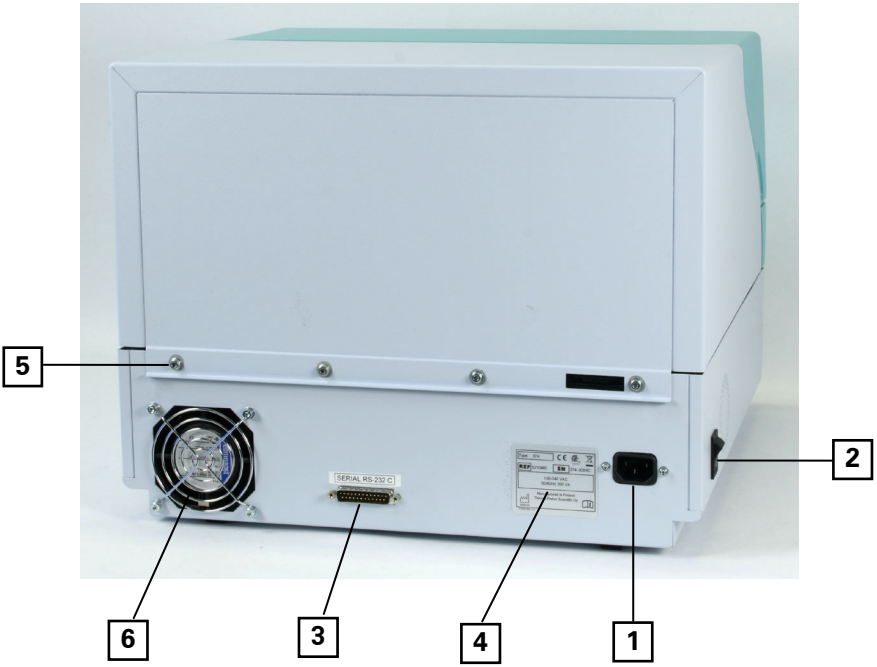
2.1 Front view



1	Instrument housing
2	Dispenser and optics cover
3	Instrument chassis
4	Measurement chamber door
5	Power, busy and error indicator

Fig. 2.1 Fluoroskan Ascent FL/Fluoroskan Ascent front view

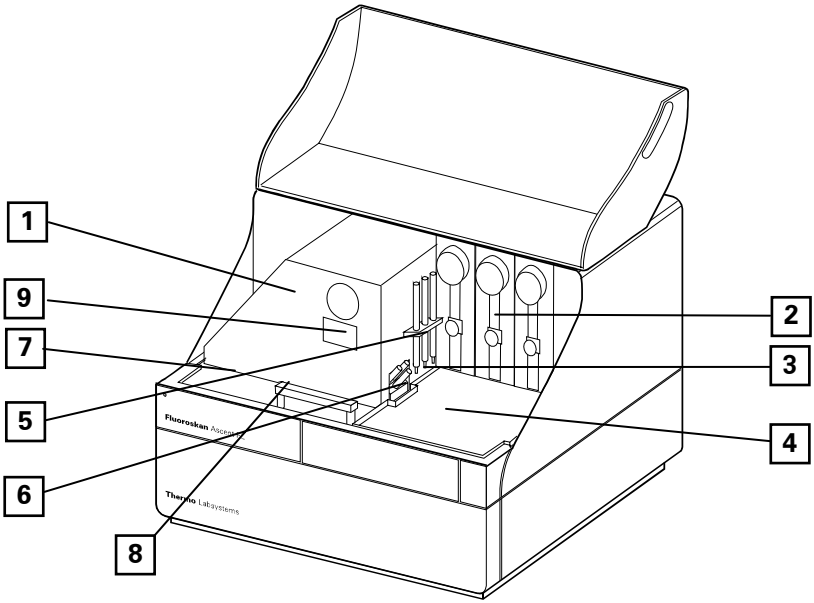
2.2 Rear view



1	Mains power supply socket
2	Power switch (ON/OFF)
3	Serial communication connector for the computer
4	Identification plate
5	Housing retaining screws, 4 pieces
6	Cooling-air outlet

Fig. 2.2 Fluoroskan Ascent FL/Fluoroskan Ascent rear view

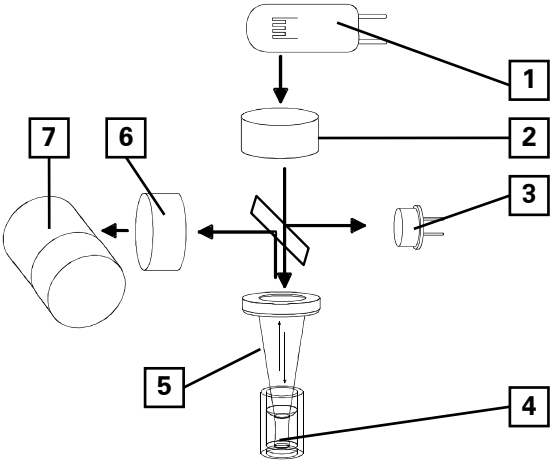
2.3 Internal view



1	Light cover for the optical unit
2	Dispensers (opt.)
3	Dispensing head (opt.)
4	Leakage tray (opt.)
5	Dispensing head holder (opt.)
6	Dummy plug
7	Control switches in the Fluoroskan Ascent FL
8	Cover sensor in the Fluoroskan Ascent FL
9	Beam selector

Fig. 2.3 Fluoroskan Ascent FL/Fluoroskan Ascent internal view

2.4 Optical system



1 The light source is a quartz-halogen lamp.

2 The excitation filter in the excitation filter wheel.

3 The lamp reference detector.

4 The highly focused excitation light beam in the well to be measured. The diameter of the normal beam in the sample is about 3 mm and that of the small beam about 1.5 mm.

5 In the Fluoroskan Ascent FL, the emission light beam is also strictly limited with a light shield to avoid crosstalk in luminometric measurements.

6 The emission filter in the emission filter wheel.

7 The photomultiplier tube detects the emission light.

Fig. 2.4 Principle of the optical system

2.5 Control switches

The control switch box (FL only) (Fig. 2.3:7) contains three rocker switches for priming and emptying dispenser tubings, one rocker switch for driving the plate carrier in or out, and a sensor (Fig. 2.3:8) to monitor that the dispenser cover is in the closed position in luminometric measurements. The priming control switches are only functional when the plate carrier is located outside the instrument.

2.6 Incubator

The incubator contains two heating element plates in the measurement chamber, one heating element plate under the microplate and another above it. The incubator only heats, but does not cool.

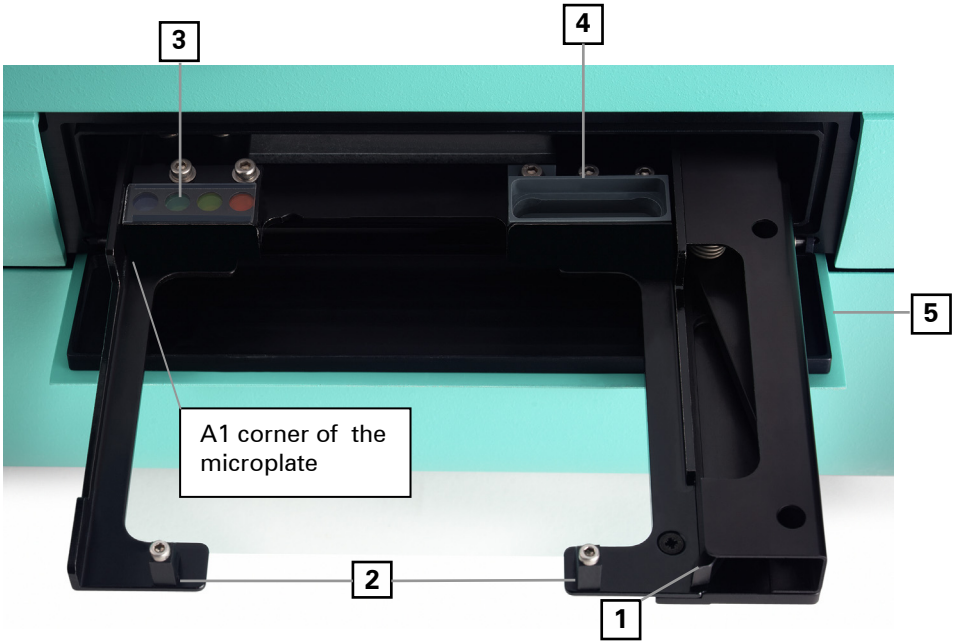
2.7 Excitation beam selector

The excitation beam selector makes it possible to select the optimal beam to measure different sizes of wells. The normal beam, diameter 3 mm, is recommended for 96-well plates and larger wells. The small beam, diameter 1.5 mm, is recommended, for example, for 384-well plates. The beam selector is situated in the optics module (Fig. 2.3:9). A manually operated knob changes the diameter of the light beam and the instrument automatically detects the position of the knob.

2.8 Dispensers

The optional dispensers, 1 to 3 dispensers from left to right, are located inside the instrument housing under the dispenser cover, as seen in Fig. 2.3. The dispensers consist of modular digital pumps with valves, syringes, tubing and dispensing heads. The dispensing heads have three alternative dispensing positions, one of these dispenses into the well in the measurement position.

2.9 Plate carrier



1 Positioning lever

2 Adjustable stoppers

3 Fluorescence reference chips

4 Waste strip holder (opt., 4 wells) for the tip priming during the measurement session

5 Plate carrier door

Fig. 2.9 Plate carrier

3. Installation

3.1 Upon delivery

3.1.1 Unpacking

The Fluoroskan Ascent FL or Fluoroskan Ascent is packed in a specially designed shipping carton. Move the unpacked 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 Fluoroskan Ascent FL or Fluoroskan Ascent instrument and accessories carefully with the arrows on the transport package pointing upwards. Open the top of the package and lift the Fluoroskan Ascent FL or Fluoroskan Ascent out of the shipping carton (Fig. 3.1). The following notes and instructions are sent with the instrument and are immediately available when you open the package:

- the packing instructions
- the packing list
- the Warranty Certificate card
- the performance test reports
- the User Manual.

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.

Caution: The Fluoroskan Ascent FL or Fluoroskan Ascent weighs approximately 21 kg (46 lbs.) without dispensers and should be lifted with care. It is recommended that two persons lift the instrument together, taking the proper precautions to avoid injury.

Retain the original packing materials and shipping carton for future transportation. Also retain all the documentation provided with the instrument.

If you relocate your instrument or ship it for service, remember to:

1. Empty the dispenser (s) and remove the tube assembly.

2. Remove any loose items from the plate carrier, for example, adapters, plates and priming vessels.
3. Remove the power cable as well as the serial cable.
4. Replace the transportation lock.

See Section 8.4 Shipping the instrument (or items) for further information.

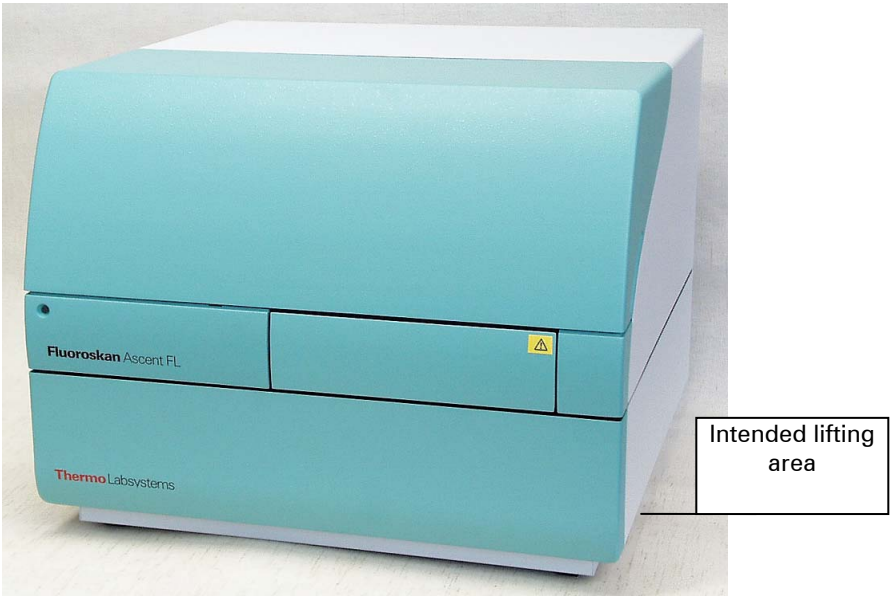


Fig. 3.1 Fluoroskan Ascent FL/Fluoroskan Ascent

3.1.2 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.

3.1.3 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*.

Visually check all interconnections in the basic instrument. Check that there are no loose parts inside the instrument.

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

3.1.4 Environmental requirements and noise

When you set up your Fluoroskan Ascent FL or Fluoroskan Ascent, 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, connections, controlling computer, printer, etc.
- 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 90% (non-condensing).

Leave sufficient space (at least 10 cm) at both sides of the instrument and at the back of the unit to allow adequate air circulation. Make space for the controlling computer on one side of the Fluoroskan Ascent FL or Fluoroskan Ascent.

The Fluoroskan Ascent FL or Fluoroskan Ascent does not produce operating noise at a level which could be harmful. No sound level measurements are needed after installation.

Warning: DO NOT operate the instrument in an environment where potentially damaging liquids or gases are present.

3.1.5 Things to avoid

DO NOT smoke, eat or drink while using the Fluoroskan Ascent FL or Fluoroskan Ascent. Wash your hands thoroughly after handling test fluids. Observe normal laboratory procedures for handling potentially dangerous samples. Use proper protective clothing. Use disposable gloves. Be sure the working area is well-ventilated.

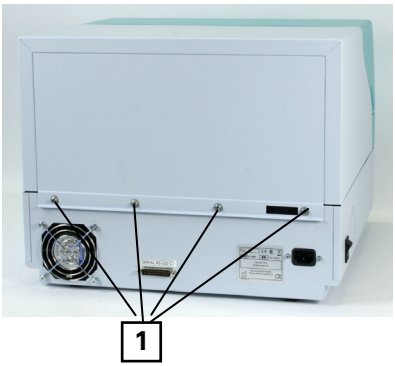
Never spill fluids in or on the equipment.

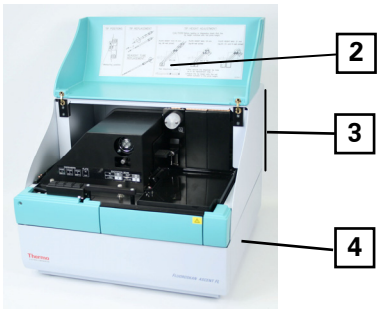
3.1.6 Technical prerequisites

Place the instrument on a normal laboratory bench close to the mains power supply socket. The net weight of the unit is approx. 21 kg (46 lbs.).

The instrument operates at voltages of 100 – 240 Vac. The frequency range is 50/60 Hz.

3.2 Releasing the transportation lock





1 Cover retaining screws, 4 pieces

2 Dispenser and optics cover

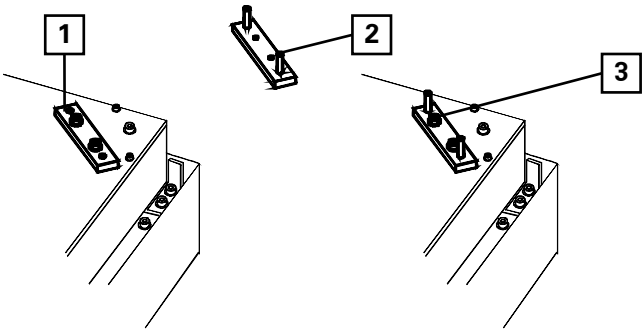
3 Rear of the cover

4 Point to where cover lifted

Fig. 3.2a Removing the instrument cover

1. Remove the four cover retaining screws (Fig. 3.2a:1).
2. Open the dispenser and optics cover (Fig. 3.2a:2).
3. Lift the rear of the cover at first about 3 cm (Fig. 3.2a:3).
4. Lift the cover aside (Fig. 3.2a:4).
5. Undo the two screws (Fig. 3.2b:1) at the right rear corner of the measurement chamber.
6. Turn the locking piece upside down (Fig. 3.2b:2).

7. Fit the locking piece back with the fitting screws (Fig. 3.2b:3).
8. Refit the cover by first fixing the front corners.



1 Connected locking piece with two screws

2 Disconnected locking piece turned upside down

3 Reconnected locking piece

Fig. 3.2b Removing the transportation lock

Caution: If the locking piece is not fitted into its place, light may enter the measurement chamber and affect the results.

3.3 Power and computer connections

1. Ensure that the power switch (Fig. 3.3:1) is in the OFF position.
2. Connect the mains supply cable to the mains power supply socket (Fig. 3.3:2). The instrument box contains two different mains supply cables with North American and European types of plugs. Select the correct type used in your laboratory. If any other type of mains supply cable is needed, use only cables certified by the local authorities.
3. Connect the instrument to a correctly installed line power outlet that has a protective conductor also called ground or earth.
4. Connect the serial cable to the serial connector (Fig. 3.3:3) and secure it with the locking screws. Connect the other end similarly to the controlling computer.

1 Power switch (ON/OFF)

2 Mains power supply socket

3 Serial connector

Fig. 3.3 Power and computer connections

Warning: Always make sure the power switch on the instrument is in the OFF position and remove the mains power supply cable from the back of the instrument prior to any installation or relocation of the instrument.

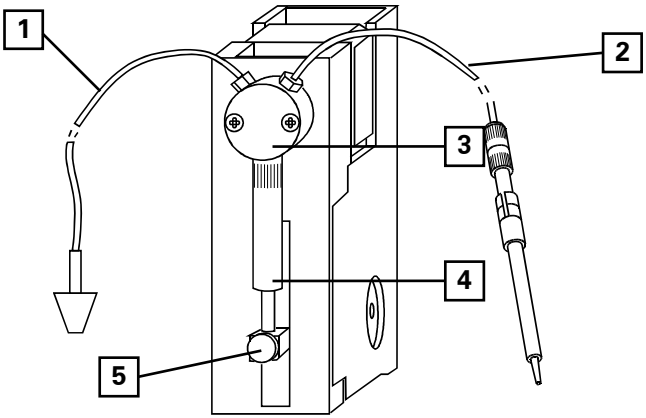
Warning: Never operate your instrument from a power outlet that has no ground connection.

3.4 Installation of dispensers

The optional dispensers 1 to 3 are installed in number order from left to right. The complete dispensing assemblies are packed with the accessories. The aspirate tubing (Fig. 3.4:1) is factory installed into the left hole of the valve. Ensure that the aspirate tubing is finger-tight. If necessary, turn the fitting another quarter to half turn using a 7.9 mm (5/16 in.) wrench. The aspirate tubing is used to fill the syringe with reagent. When using the dispensers, make sure that the aspiration tube end is completely submerged in the reservoir and there is a sufficient volume of the reagent in the reservoir (for all primings and actual dispensing).

Fit the complete dispensing tube assembly (Fig. 3.4:2) into the right hole of the valve and tighten it finger-tight. Then turn the fitting another quarter to half turn using a 7.9 mm (5/16 in.) wrench. The dispensing tube is used to dispense reagent from the syringe into a microplate. Place the dispensing heads in the dispensing head holder on the left-hand side of the dispensers.

First push the plunger manually upwards into the upper position before tightening the plunger lock screw (Fig. 3.4:5). Ensure that the plunger lock screw is sufficiently tightened. Note that the plunger can be extremely stiff!



1 Aspirate tube assembly (incl. tubing and end weight)

2 Complete dispensing tube assembly

3 3-port valve

4 Dispensing syringe (1.0 ml) and plunger

5 Plunger lock screw

Fig. 3.4 Automatic dispenser unit

3.5 Installation of the drop plate

The instrument is supplied with a special drop plate (Fig. 3.5a). The drop plate is used to protect the instrument from damage caused by accidental dispensing without any microplate. If the user forgets to place a microplate onto the plate carrier but has the drop plate in place, the reagent will be dispensed into the drop plate, not inside the instrument. The drop plate is placed like an adapter into the plate carrier (Fig. 3.5b) and the microplate is placed onto the drop plate. The holding capacity of the drop plate is 19 ml of liquid.

Note: The drop plate cannot be used for bottom reading.

Fig. 3.5a Drop plate

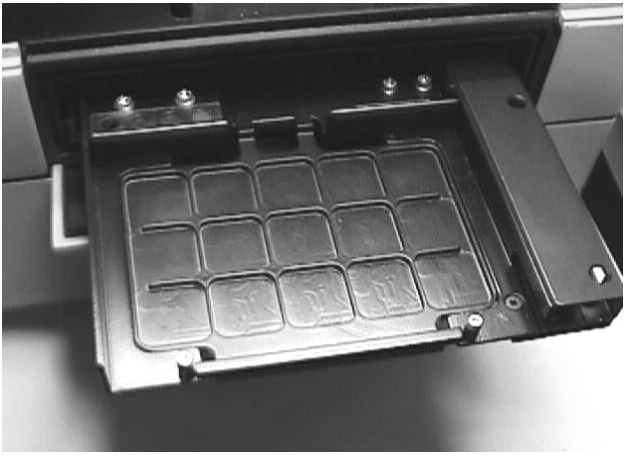


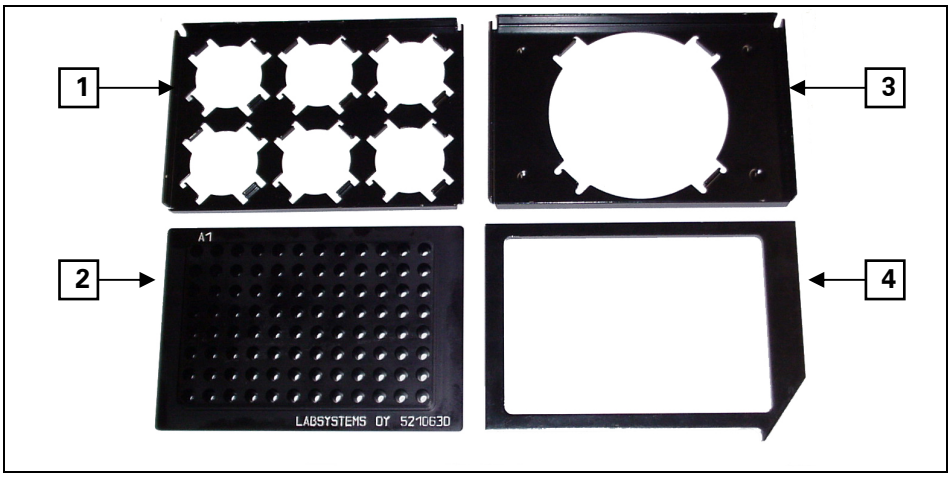
Fig. 3.5b Drop plate placed into the plate carrier

3.6 Plate adapters

Reading certain plate types with the Fluoroskan Ascent FL and Fluoroskan Ascent instruments require special plate adapters (Fig. 3.6). These plate adapters are required for the following reasons:

1. Raising the 384-well plate with a height of less than 15 mm to the proper height for luminometric measurement.
2. If the 384-well plate height is less than 13.5 mm, an adapter must be used to raise the plate for dispensing.
3. Positioning of other sample vessel types than microplates (for example, Petri dishes or Terasaki plates) onto the plate carrier for measurement and dispensing.
4. Supporting flexible PCR plates and tubes on the plate carrier for measurement and dispensing.

When using any adapter, measure the height of the plate and the adapter together and ensure that this value is correctly entered into the Ascent Software plate template parameter file. Refer to the Thermo Scientific Ascent Software User's Guide for further information.



1 Adapter for Petri dish, 6 x 40 mm

2 Adapter for 96-well PCR plates

3 Adapter for Petri dish, 93 mm

4 Adapter for low 384-well plates

Fig. 3.6 Example of plate adapters

Note: If the orientation of the plate adapter is important, the correct orientation is printed on the adapter or the adapter has been designed in such a way that only one orientation is possible.

When the adapter is needed, place it into the plate carrier. Then place the sample vessel(s) on the corresponding adapter, select the correct plate template (marked "with adapter") from Ascent Software and run the instrument normally.

Note: Always remember to remove the plate adapter before using the instrument with any other plate types.

3.7 Adjusting the plate carrier

The plate carrier of the Fluoroskan Ascent FL and Fluoroskan Ascent has been designed for plates with different footprints and to be robot compatible. The plate carrier has two adjusting knobs with two different orientations. These knobs have been set for standard plate types at the factory. The setting is also valid for the robotic 384-adapter but readjustment of the knobs will be needed with the plate types requiring special adapters (see Section 3.6). Fig. 3.7 shows how the knobs should be adjusted for special plate adapters. Both knobs should be rotated 90° clockwise after which all special adapters fit into the plate carrier.

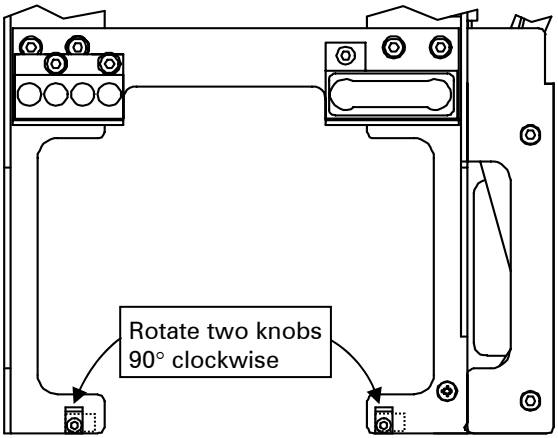


Fig. 3.7 Two plate carrier knobs rotated 90° clockwise for special plate adapters

3.8 Operational check

Switch the instrument ON. The instrument automatically performs a complete set of initialization tests or adjustments.

When the initialization tests and adjustments have been completed successfully, the indicator LED turns from yellow to green.

If anything fails in the initialization tests or adjustments, the indicator LED will turn red. In this case, switch the instrument OFF and ON again. If this failure is repeated, contact authorized technical service.

The instrument is ready for operation.

The instrument also performs different kinds of additional runtime hardware tests before each measurement run. Autocalibration (in fluorometry) and automatic blanking (in luminometry) are performed.

Because of the relative nature of fluorometry and luminometry, we recommend you use known samples or controls to verify instrument operation.

4. Operation

The Fluoroskan Ascent FL/Fluoroskan Ascent is fully computer-controlled. Ascent Software controls the reader functions and provides complete data handling and report formatting. For further details on how to use the software, refer to the Ascent Software User's Guide.

Warning: DO NOT use any other software than Thermo Scientific software designed for this instrument. Use only valid combinations of the PC software and the instrument's internal EEPROM.

Warning: The Fluoroskan Ascent FL/Fluoroskan Ascent instruments do not verify the logic flow of the received commands.

The instrument is equipped with a power switch (ON/OFF) and a three-color LED indicator. When the instrument is switched ON, the color indicates the state of the instrument:

Green	The instrument is ready and waiting for a command.
Orange	The instrument is busy, executing a command.
Red	The instrument has found an error, the error message is sent to the computer and the computer has not acknowledged it.

4.1 Switching on

When switched on, the instrument performs the following initialization tests and adjustments:

1. The measurement system is tested using an internal test signal, then different gain steps are tested and adjusted.
2. Both filter selection systems are tested and the filters in position 1 are selected.
3. X and Y movements of the plate carrier are tested and the carrier is driven out.

4. When all the initialization tests and adjustments have been performed successfully, the indicator LED turns green and the instrument is ready to receive computer commands. The recommended warm-up time is 15 minutes, but the instrument will perform commands immediately after the initialization period.

4.2 Loading the microplate

The microplate is loaded onto the instrument plate carrier for measurement. The plate carrier is able to handle microplates of different sizes, therefore the free space in the carrier is clearly larger than the standard 96-well plate. The positioning lever in the plate carrier (Fig. 4.2) will automatically position the plate correctly into the upper left corner of the carrier when the plate is driven in.

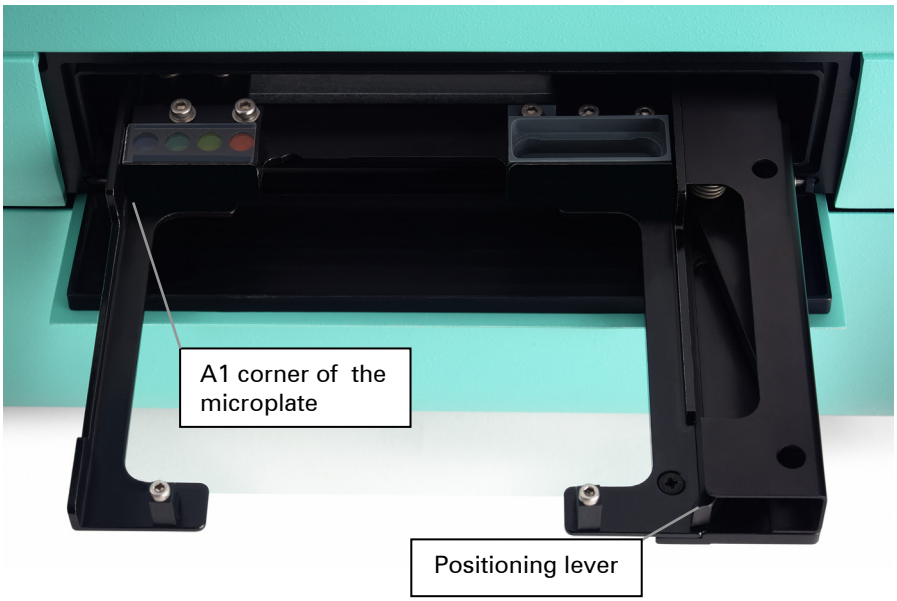


Fig. 4.2 Plate carrier

4.3 Fluorometric measurement

A measurement function has several phases:

1. The instrument switches on the halogen lamp and waits for 5 seconds to balance the intensity if the lamp was off. The lamp is switched off if the instrument is idle and has not received any commands from the computer in 3 minutes.
2. The anode voltage of the PMT is selected according to the excitation and emission wavelengths to compensate for the spectrum of the halogen lamp and the spectral response of the photomultiplier tube.
3. Selected filters are driven to the measurement positions by rotating the excitation and emission filter wheels.
4. The plate carrier is driven in and the reference chip corresponding to the selected filter pair is driven to the measurement position.
5. In the autocalibration procedure the instrument reads the fluorescence from the reference chip, compares it to the value in memory and sets a factor to correct the reading. The original chip value is in memory and due to the drift of the PMT and the drift of the lamp, the measured chip value may differ slightly from the original one. This difference is used to correct the reading in order to obtain the original signal levels. Also see Section 4.3.1 Validation. In long measurement procedures autocalibration is performed in a suitable phase without disturbing the measurement timing. The time interval between autocalibrations is 5 minutes.
6. After autocalibration the wells are measured according to the selected measurement parameters. The A/D converter has several gain steps, but the instrument automatically selects the best gain in each measurement.
7. The measurement is performed with a selected integration time. The integration time can be from 20 to 1000 ms, which defines the length of time used to obtain one measurement result. The minimum time is 20 ms (default) during which time the instrument sums 150 readings from the A/D converter.
8. If the integration time is longer, the result is the mean value of the number of 20 ms readings during the total integration time. Longer than 20 ms integration times can be used to reduce background noise if the signal level of the sample is very low. With small Stokes shift values, optical crosstalk of excitation and emission filters may dominate the

instrument background noise. In these cases an extended integration time does not help. The plate carrier acceleration and maximum speed are adjustable, see the Ascent Software User's Guide: ***General*** step /***Settings***.

Caution: When measuring large wells with low viscosity samples, high speed and acceleration may cause splashing and extra variation in the results. Use the default acceleration 2 for 48-well plates and the default acceleration 1 for microplates with 24 or less wells.

Note: Ensure that there are no items preventing the closure of the plate carrier door when measurement is started. DO NOT open the plate carrier door during measurement.

4.3.1 Validation

1. In the validation procedure a new filter pair is introduced to the autocalibration system. The Fluoroskan Ascent FL/Fluoroskan Ascent has a special automatic gain selection to obtain the optimal dynamic range without any adjustments. The automatic gain selection optimizes the relation between the excitation and emission wavelengths and the anode voltage of the photomultiplier tube to compensate for the halogen lamp intensity spectra as well as PMT spectral response. The validation procedure always uses the normal beam only. You can find the filter pair validation procedure in Ascent Software. The menu selection is ***Setup***/***Filters***.
2. The instrument reads the fluorescence values of all the reference chips, selects the chip for which the value is suitable, neither too low, nor too high. The selected value is saved in memory for the autocalibration procedure.
3. If the validation procedure does not find a suitable reference chip, the program requests if the user still wants to use this filter pair. If the answer is "Yes", the pair can be used in measurements. The only difference is that the autocalibration procedure cannot form a correction factor to compensate for the drifts mentioned in the previous section. Other compensations, such as the excitation intensity compensation with the reference channel, remain in the autocalibration procedure.
4. Ascent Software shows the validated filter pairs, and those pairs which have not found a suitable chip are marked with "*".

4.3.2 Scaling

The scaling factor is a value that multiplies all the readings from the instrument. It is defined separately for each filter pair (fluorometry) or filter position (luminometry). The scaling factor can be used to convert the instrument reading to a desired level or to adjust several instruments to give similar signal values.

1. The measured results are expressed as Relative Fluorescence Units (RFU). Scaling is a way to convert readings to show desired values. The normal beam and the small beam have separate scaling factors.
2. Prepare a scaling reference solution and a blank solution and pipette several wells of blanks and the known concentration.
3. Measure the wells using the correct filter pair and beam.
4. Calculate the average values of the measured reference values and blank values.
5. Calculate the scaling factor using calculated average values:

Factor = Known reference / (Measured reference - Measured blank)

Example

- The known concentration is 500 pmol/well.
- The calculated average of the measured reference wells is 1825 RFU.
- The calculated average of the measured blank wells is 0.2 RFU.
- Factor = 500 / (1825 - 0.2) = 0.274.
- After scaling the measured result is: 0.274 x 1825 RFU = 500 pmol/well.

You can find the filter pair scaling entry in Ascent Software. The menu selection is ***Setup*/*Filters***. Select the corresponding filter pair and key in the scaling factor.

The following filter pairs, if any of these are factory installed in the instrument, are scaled as follows:

Filter pair	*Scaling reference*	*Scaled reading (approx.)*
Ex 355 nm, Em 460 nm	1000 pmol 4-Methylumbelliferone, free acid (4-MeU), Sigma M-1381	1000
Ex 485 nm, Em 518 nm, 527 and 538 nm	10 pmol Fluorescein, Sodium salt, Sigma F-6377	10
Ex 544 nm, Em 590 nm	625 pmol Tetramethylrhodamine isothiocyanate (TRITC), Sigma T-5646	625
Ex 584 nm, Em 612 nm	500 pmol Texas Red, Sigma S-3388	500

Ex = excitation filter; Em = emission filter

Note: The scaling factors used can be seen from the instrument status report.

4.3.3 Dynamic series test solutions

Dynamic series for the Fluoroskan Ascent FL/Fluoroskan Ascent filter pair excitation filter 485 nm/emission filter 538 nm.

Fluorescein (Sigma F-6377, M = 376.3 g/mol, store at amb. temp.).

Reagents

0.01 M PBS, pH 9.0 (dilution buffer):

$K_2HPO_4 \cdot 3\ H_2O$	(M = 228.23 g/mol)		2.28 g
NaCl	(M = 58.44 g/mol)		9.0 g
Distilled water		ad.	1000 ml

Adjust the pH if necessary with a few drops of 0.1 M NaOH to pH 9.0.

25 mmol/l Fluorescein (stock solution)

Fluorescein, Sodium salt (Sigma F-6377, M = 376.3 g/mol)		94 mg
Pure ethanol 94%		3 ml
0.01 M PBS, pH 9.0	ad.	10 ml

Dilution series

No.	*pmol/100 µl*	*Make the dilutions in 10 ml tubes as follows:*			
13	5000	100 µl Fluorescein (stock sol.)	ad. 50 ml	0.01 M PBS, pH 9.0	
12	1000	2 ml dilution 13	ad. 10 ml	"	(+ 8 ml)
11	100	1 ml dilution 12	ad. 10 ml	"	(+ 9 ml)
10	50	3 ml dilution 11	ad. 6 ml	"	(+ 3 ml)
9	10	1 ml dilution 11	ad. 10 ml	"	(+ 9 ml)
8	5	3 ml dilution 9	ad. 6 ml	"	(+ 3 ml)
7	1	1 ml dilution 9	ad. 10 ml	"	(+ 9 ml)
6	0.5	3 ml dilution 7	ad. 6 ml	"	(+ 3 ml)
5	0.1	1 ml dilution 7	ad. 10 ml	"	(+ 9 ml)
4	0.05	3 ml dilution 5	ad. 6 ml	"	(+ 3 ml)
3	0.025	2 ml dilution 5	ad. 8 ml	"	(+ 6 ml)
2	0.01	1 ml dilution 5	ad. 10 ml	"	(+ 9 ml)
1	0.005	3 ml dilution 2	ad. 6 ml	"	(+ 3 ml)
BLANK		0.01 M PBS, pH 9.0			

Pipetting

Use a Black Microtiter Strip plate (Cat. no. 95029450)

Blank: 100 µl/well in eight replicates into column 1

Dilutions: 100 µl/well dilutions 1 – 9 in eight replicates into columns 2 – 10

100 µl/well dilutions 10 – 13 in four replicates into columns 11 – 12.

4.4 Luminometric measurement

Only the Fluoroskan Ascent FL can measure luminometric labels. Luminometric reading is optimized for 96- and 384-well plates and top reading. To obtain the best sensitivity and the lowest crosstalk, a light shield (Fig. 4.7a) between the optics and the plate must be used. The light shield does not interfere in fluorometric measurements. In luminometric measurements the dispenser cover must be closed.

Reading 1- to 48-well plates as well as bottom reading can cause increase in background signal and effect sensitivity in luminometric measurements, because the light shield cannot be used and most of these plates are transparent, which enables light leakage from one well to another.

A measurement function has several phases:

1. The anode voltage of the PMT is set according to the selected value of the software.
2. The excitation filter wheel is driven in between the two filter positions and the excitation light is blocked even when the light is off. The emission filter slot 8 (= block) is held in place until the plate door is closed. The empty position/selected filter is then driven to the measurement positions. The emission filter slot 8 is also used for the blanking procedure to compensate for the possible PMT drift.
3. In the blanking procedure the instrument reads the PMT dark signal. If the bottom value is drifting, the results are compensated. To obtain the most accurate results, the measurement time of the dark must be as long as the measurement time of the sample. The measurement time of the dark is divided into two parts. The first half is measured before the sample measurement and the second half after the sample measurement. The time used for the instrument blanking procedure can be selected from Ascent Software (refer to the Overview part of the Ascent Software User's Guide).

Note: Ensure that there are no items preventing the closure of the plate carrier door when measurement is started. DO NOT open the plate carrier door during measurement.

4.4.1 Luminometric scaling

The measured results are expressed as Relative Light Units (RLU). Scaling is performed similarly as in fluorometric scaling, see Section 4.3.2 Scaling.

4.5 Other functions

4.5.1 Orbital shaking

The track movement system can perform the shaking action. The speed is adjustable from 60 to 1200 rpm (revolutions per minute) and the diameter of the orbital movement is adjustable from 1 to 50 mm.

Some combinations of speed and diameter would cause too high g-forces inside the well area resulting in spills inside the measurement chamber. Therefore, only certain combinations are available. When you use plates with small wells, such as plates with 48 wells or more, you can select any of these available speed and diameter combinations to serve your application best (Table 4.5.1a).

The following tables (Table 4.5.1a – Table 4.5.1e) show the recommended and the not recommended but available and unavailable speed and diameter combinations with different plate types. These tables are based on the liquid used being of low viscosity like water and the volumes being appropriate (the wells are not full). The gray area in the tables show the recommended (Recommended), the black area the not recommended (Possible), and the white area the prevented speed and diameter combinations (Not allowed).

Table 4.5.1a

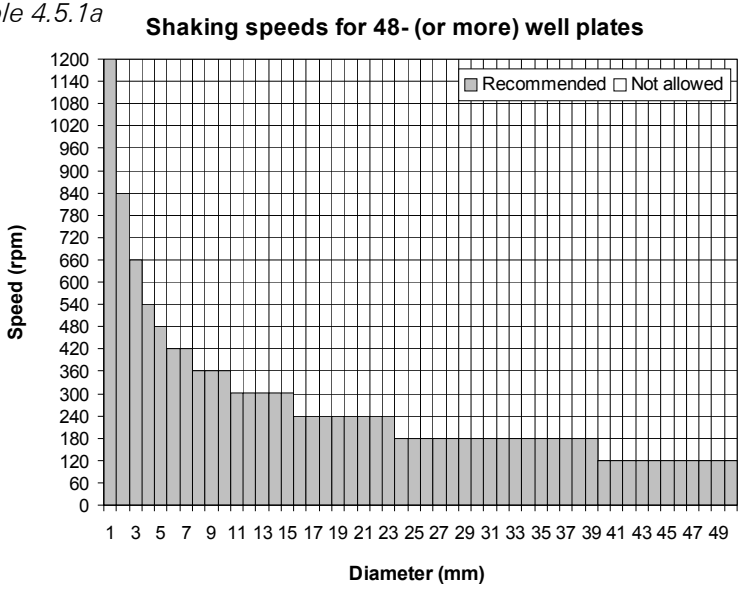


Table 4.5.1b

Shaking speeds for 24-well plates

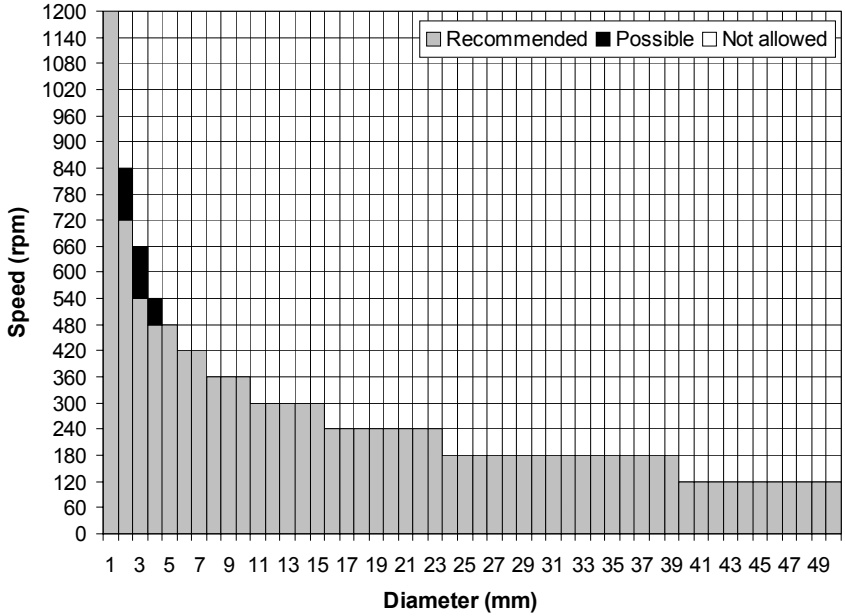


Table 4.5.1c

Shaking speeds for 12-well plates

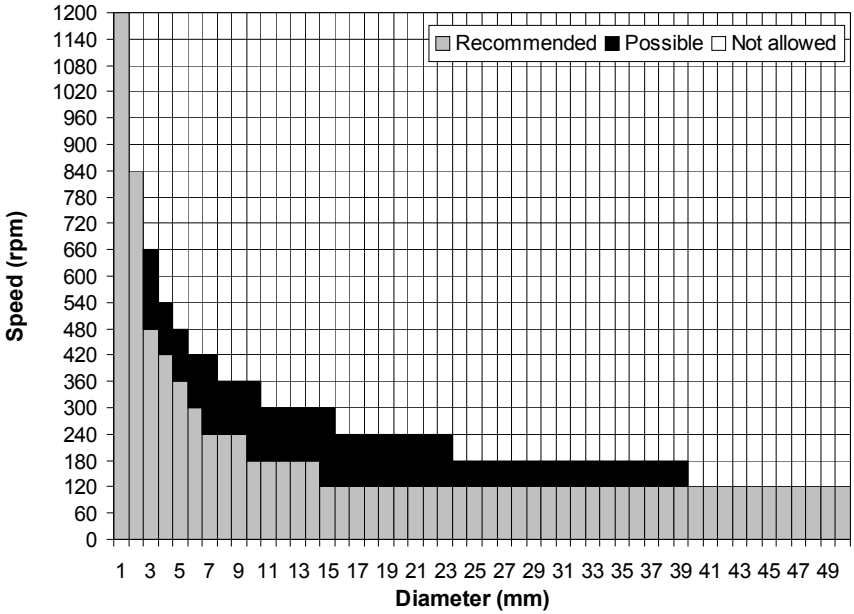


Table 4.5.1d

Shaking speeds for 6-well plates

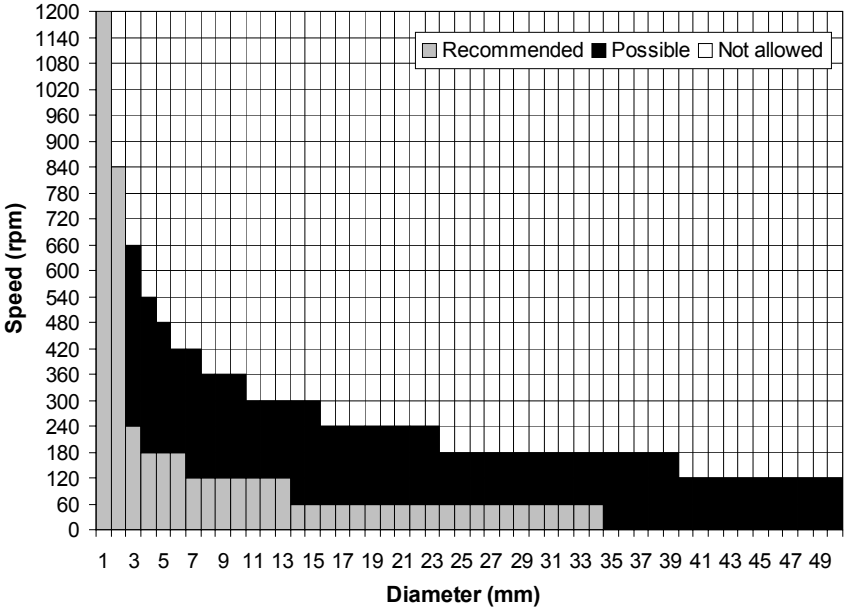
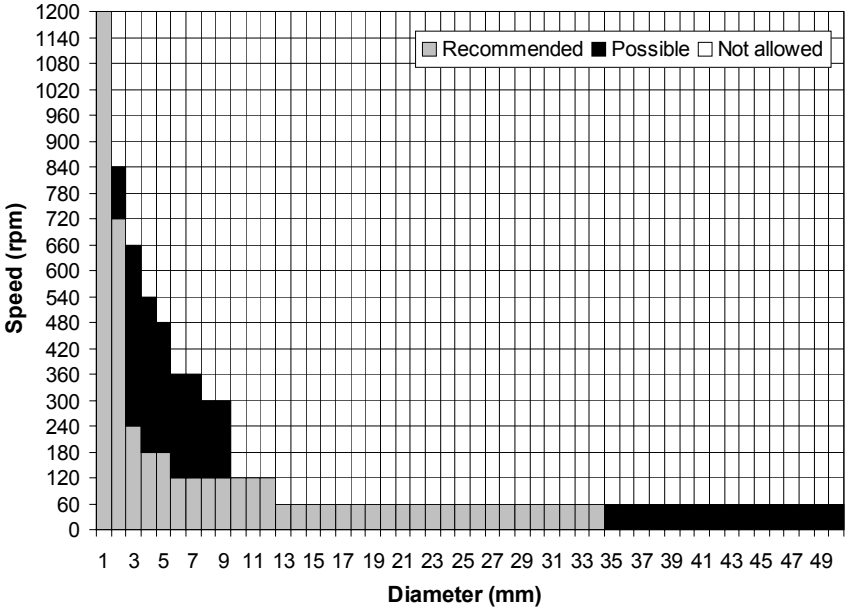


Table 4.5.1e

Shaking speeds for 95 mm Petri dish



4.5.2 Incubator

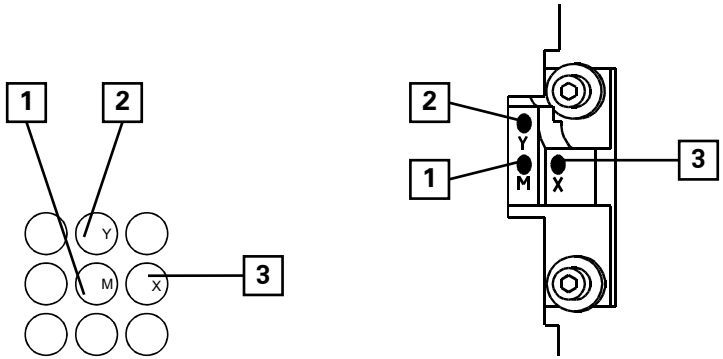
The incubator contains two heating element plates in the measurement chamber, one heating element plate under the microplate and another above it. Both heating element plates are temperature-controlled. The upper plate is slightly warmer than the lower plate to avoid condensation on the plate lid. The measurement chamber is large to adopt different plate formats and therefore extensive evaporation may cause some variations in the temperatures between the wells. Consequently, when using incubations with extended periods of time in the instrument, a plate lid is recommended.

If the incubation period is long without any other important functions, the Fluoroskan Ascent FL/Fluoroskan Ascent automatically changes the place of the plate within the measurement chamber to minimize temperature differences between the wells.

4.5.3 Dispensers

The optional dispensers, 1 to 3 dispensers numbered from left to right, are located inside the instrument housing under the dispenser cover (Fig. 2.1:2). The dispensing heads have three alternative dispensing positions, one of these dispenses into the well in the measurement position (M). The positions are optimized for a 96-well plate (Fig. 4.5.3a). When the dispensers are not in use, the dispensing heads may be stored in the dispensing head holder, but the tip holes to the measurement chamber must be closed with dummy plugs.

Note: Ensure that the tip position in the instrument corresponds to that defined in Ascent Software.



1 *M position*: Dispensing head directed in the measurement well position. Dispensing into the well during the measurement step is possible with this position.

2 *Y position*: Dispensing head directed in the well next to the measurement position in the Y direction. When this position is used for dispensing, an extra plate movement is carried out before the measurement step causing minor time delays.

3 *X position*: Dispensing head directed in the well next to the measurement position in the X direction. When this position is used for dispensing, an extra plate movement is carried out before the measurement step causing minor time delays.

Fig. 4.5.3a Dispenser tip positioning optimized for a 96-well plate

Before inserting the dispensing head into a dispensing position, prime the syringe and tubing to an external priming vessel. The instrument has no internal priming vessel for priming the syringe and tubing. You can find the priming instructions in the Ascent Software User's Guide. The minimum priming volume needed is 700 µl and the recommended volume is 2700 µl.

The Fluoroskan Ascent FL also has control switches for priming the dispenser tubing. With these switches, priming can be carried out alternatively by using an external priming vessel or by using an empty microplate on the plate carrier as a priming vessel. When priming is performed with the dispensing heads installed into the M, X or Y positions, place the empty priming vessel into the plate carrier. Initiate priming by pressing the corresponding switch when the plate carrier is located outside the unit. Priming is carried out as long as the corresponding switch is pressed.

Note: The priming control switches are functional only when the plate carrier is located outside the instrument. DO NOT use priming vessels higher than the actual plate intended to be used in the assay. Notice the dispensing height adjustment.

Priming with Ascent Software must always be performed with the dispensing heads removed from the M, X or Y dispensing positions.

Note: Never use liquids that can cause any precipitation, clotting or contain any mechanical particles with the automatic dispensers.

The instrument has a Prime Tip feature. If this function is selected in the ***Dispense*** or ***Dispense And Measure*** steps in Ascent Software, the dispenser dispenses about 5 µl reagent into the tip priming vessels every time the instrument fills the syringe. This makes the volume of the first well equal to that of the others. We recommend you use the tip priming feature when the dispensing volumes are small, for example, 5 – 20 µl.

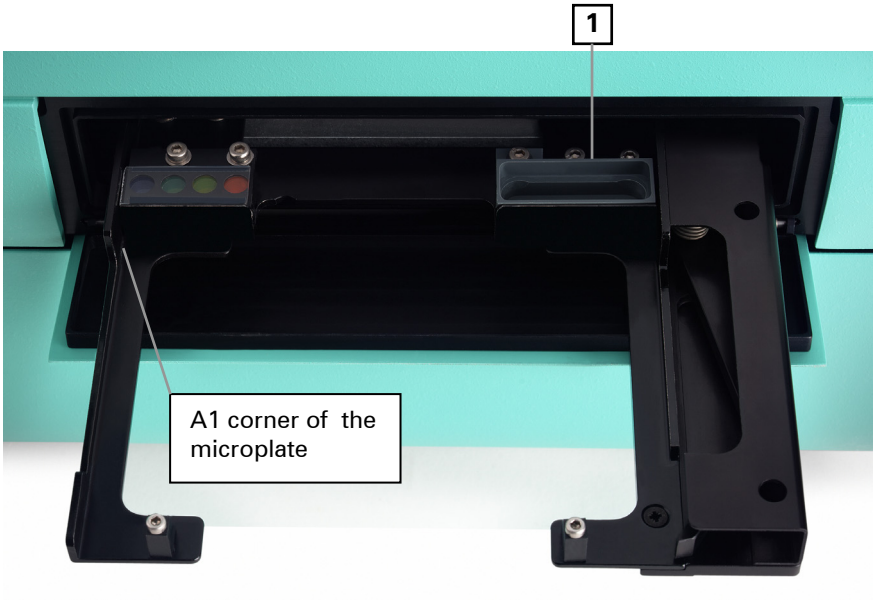


Fig. 4.5.3b Tip priming vessel (1) is a piece of a breakable strip

The recommended tip priming vessel consists of four wells of a breakable 96-well plate strip (Fig. 4.5.3b). There is a holder for the tip priming vessel in the right rear corner of the plate carrier. The four-well piece of a strip should be exchanged after about 300 tip primings.

You may need to adjust the dispenser speed. The default setting is for a water-based liquid. You can find the adjustments and selections in Ascent Software.

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

4.5.4 Dispenser head height adjustment

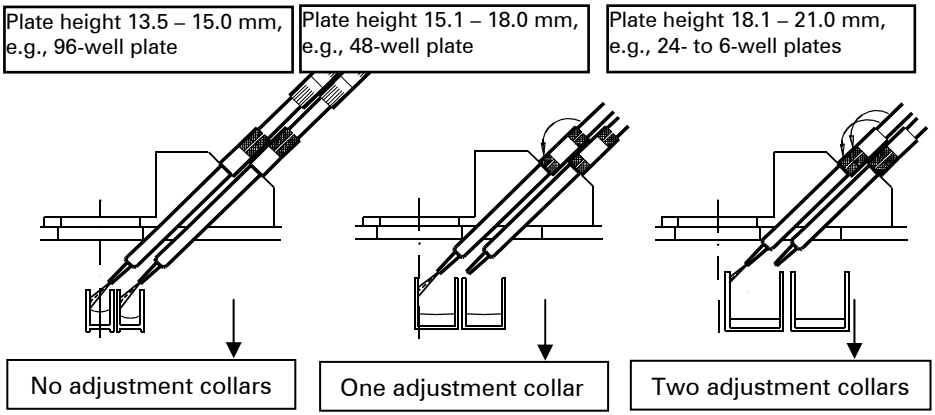


Fig. 4.5.4a Dispenser head height

The correct dispenser head height (Fig. 4.5.4a) is very important to avoid contaminating neighboring wells. The correct tip height is also important to prevent damaging the tip or the plate.

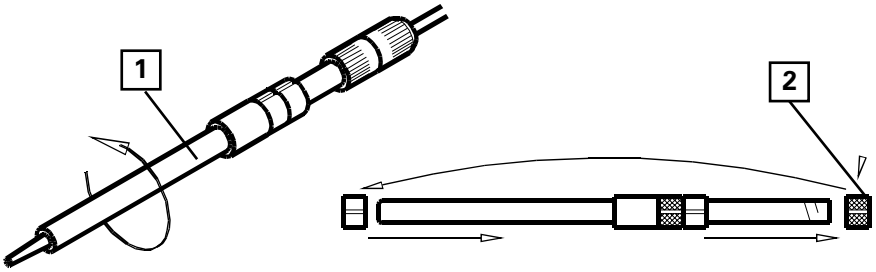
Note: Ensure that the dispenser tips are always correctly inserted sufficiently deep into their slots.

The plate height is one of the template parameters in Ascent Software and it is defined as the height of the uncovered well from the bottom of the plate, not the inside height of the well. The selected dispenser head height, the used plate and the template selected in Ascent Software must match to avoid problems. To see the heights of plates in Ascent Software, select ***Setup*** and edit ***Plate Templates***.

Some 384-well plates are lower than the standard 96-well plates. If the plate height is less than 13.5 mm, an adapter must be used to raise the plate. Measure the height of the plate and the adapter together and enter this value to the plate template.

Caution: Plate manufacturers may change the dimensions of plates without any prior notice or change in order numbers. Check the dimensions when you start using plates from a new box.

The dispenser head height is adjusted with red adjustment collars by moving them from either side of the fixed stopper collar (Fig. 4.5.4b).



1 Dispenser head tube

2 Red adjustment collars

Fig. 4.5.4b Changing the position of the red adjustment collars

1. Remove the dispenser head tube (Fig. 4.5.4b:1) from the brass tube lock.
2. The red adjustment collars (Fig. 4.5.4b:2) should be moved from one side of the fixed collar to the other to select the correct dispenser head height.
3. Fit the dispenser head tube back into place.

4.5.5 Chemical resistance of the dispenser

The following table (Table 4.5.5) is intended to provide guidelines for compatibility with materials used in the fluid path of the dispensers. 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, may result in damage to the dispenser and/or test results.

Plastic materials used in dispensers:

Polysulfone: Cross Flow Manifold Assembly in the aspirate syringe
Teflon (PTFE, TFE, FEP): tubing; valve plug, and seal
Kel F: valve body
Polypropylene: fittings for tubing, and dispensing tip

Note: 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

Table 4.5.5 Compatibility chart of materials suitable with the dispenser

Solvent	***Polysulfone***	***Teflon***	***Kel F***	***Polypropylene***
Acetaldehyde	–	0	0	0
Acetates	–	–	0	0
Acetic Acid	0	0	0	0
Acetic Anhydride	–	–	0	–
Acetone	3	0	0	0
Acetyl Bromide	–	0	–	–
Ammonia	0	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	3	0	3	*
Benzyl Alcohol	–	0	0	0
Boric Acid	–	0	0	0
Bromide	–	0	0	*
Butyl Alcohol	2	0	0	1
Butyl Acetate	3	0	–	*
Carbon Sulfide	–	0	–	*
Carbon Tetrachloride	0	0	1	3
Chloroacetic Acid	–	0	0	–
Chlorine	–	0	1	3
Chlorobenzene	3	–	–	3
Chloroform	3	0	–	3
Chromic Acid	3	0	0	–
Cresol	–	0	–	*
Cyclohexane	0	0	–	3
Dimethyl Sulfoxide (DMSO)	0	0	0	0
Ethers	–	0	–	**
Ethyl Acetate	3	0	–	0
Ethyl Alcohol	0	0	–	0
Ethyl Chromide	–	0	1	3

Continued

Solvent	***Polysulfone***	***Teflon***	***Kel F***	***Polypropylene***
Formaldehyde	0	0	0	0
Formic Acid	–	0	0	0
Freon	2	0	2	0
Gasoline	2	0	0	3
Glycerine	0	0	0	0
Hydrochloric Acid	0	0	0	0
Hydrochloric Acid (conc.)	0	0	0	0
Hydrofluoric Acid	2	0	0	*
Hydrogen Peroxide	–	0	0	0
Hydrogen Peroxide (conc.)	–	0	0	0
Hydrogen Sulfide	–	0	0	0
Kerosene	2	0	0	0
Methyl Ethyl Ketone (MEK)	3	0	–	0
Methyl Alcohol	0	0	–	0
Methylene Chloride	3	0	0	3
Naphtha	0	0	1	0
Nitric Acid	0	0	0	0
Nitric Acid (conc.)	3	0	0	–
Nitrobenzene	–	0	–	**
Phenol	–	0	–	0
Pyridine	3	0	–	–
Silver Nitrate	–	0	–	0
Soap Solutions	–	0	–	0
Stearic Acid	–	0	–	*
Sulfuric Acid	0	0	0	0
Sulfuric Acid (conc.)	3	0	0	–
Sulfurous Acid	–	0	0	0
Tannic Acid	–	0	0	0
Tannin Extracts	–	–	–	–
Tartaric Acid	–	0	–	–
Toluene	3	0	1	**
Trichloroethylene	3	0	3	3
Turpentine	2	0	0	**
Water	0	0	0	0
Xylene	3	0	0	*

* Polypropylene – satisfactory to 22°C (72°F), ** Polypropylene – satisfactory to 49°C (120°F)

4.5.6 Excitation light beam selector

Excitation beam selection means a possibility to select the normal beam, with a diameter of 3 mm in the measurement well, or the small beam, with a diameter of 1.5 mm. The small beam is required to measure, for example, 384-well plates. If the normal beam is used with 384-well plates, the light beam also excitates the walls of the well, and the result is not optimal. Measurement of larger wells with the small beam is possible and depends on the application.

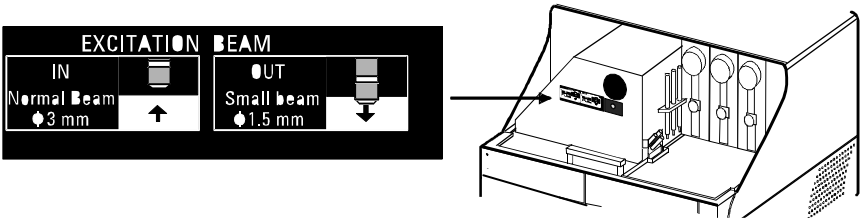
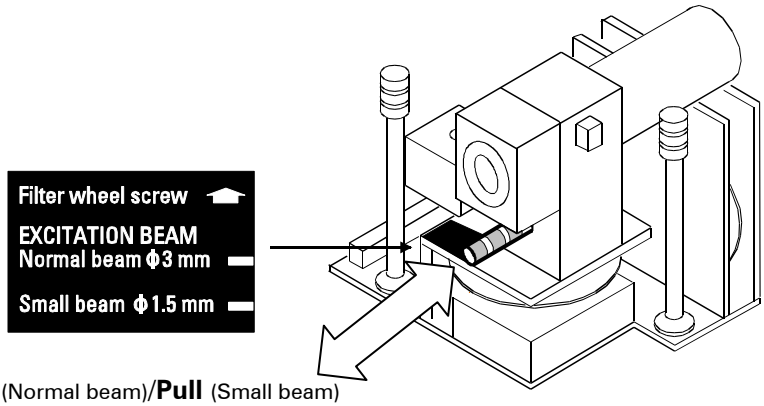


Fig. 4.5.6a Beam selection when carrying out top reading

The selection lever of the beam is on the front panel of the optical unit. When the optical unit is above the plate, the selection knob can be seen through the hole in the light cover of the optical unit (Fig. 4.5.6a).

When the optical unit is below the plate, the measurement chamber must be lifted up to reach the selection knob (see Section 4.6 Changing the measurement direction points 1 – 6). The position of the selection knob in the optical unit is shown in Fig. 4.5.6b.



Push (Normal beam)/**Pull** (Small beam)
(a pointer shows which beam is on)

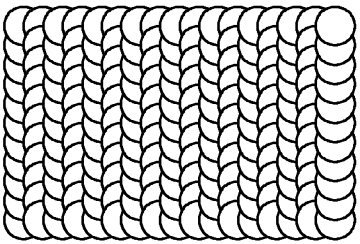
Fig. 4.5.6b Beam selection when carrying out bottom reading

To select the normal beam, push the button firmly. To select the small beam, pull the button firmly.

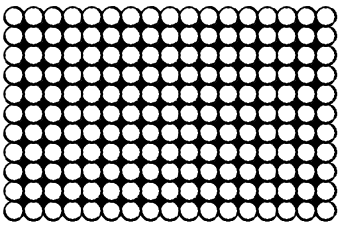
Caution: DO NOT leave the button in a middle position. The pointer on the knob also shows the position of the selection.

4.5.7 Area measurements

In area measurements (Fig. 4.5.7), when there is more than one measurement point in a well, we recommend you use the normal beam. The distance between two measurement positions is 1.5 mm. When you use the normal beam (Ø 3 mm), the measurement areas overlap and the whole area is measured. If you use the small beam (Ø 1.5 mm), the small areas between the measurement points are not measured at all.



Area measurement with the **normal beam**. The whole area is measured. This is the *recommended* way.



Area measurement with the **small beam**. The black area between the measurement points is not measured at all.

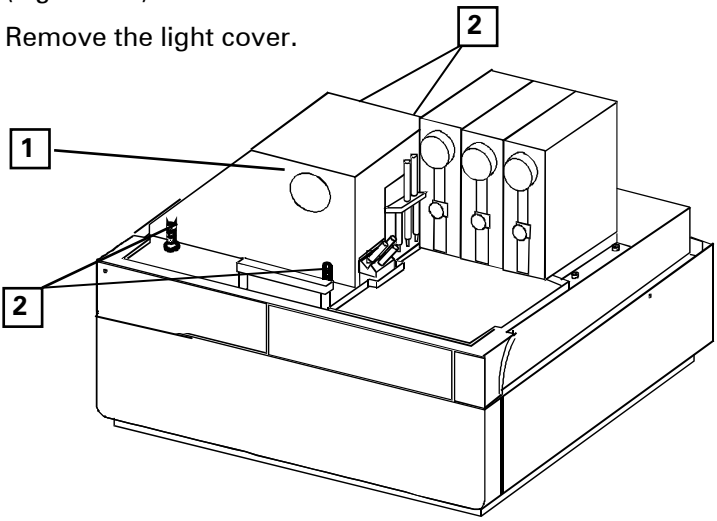
Fig. 4.5.7 Area measurements

4.6 Changing the measurement direction

The measurement direction can be changed by moving the whole optical unit from above the measurement chamber to below the measurement chamber, or vice versa.

1. Switch off the instrument and disconnect the mains power supply cable (Fig. 3.3:2). There are anode voltages inside the mains power supply box and the optical unit if the power is on.
2. Remove the instrument cover as described in Section 3.2 Releasing the transportation lock.

3. Undo the four finger nuts (Fig. 4.6a:2) to release the light cover (Fig. 4.6a:1).
4. Remove the light cover.



1 Light cover

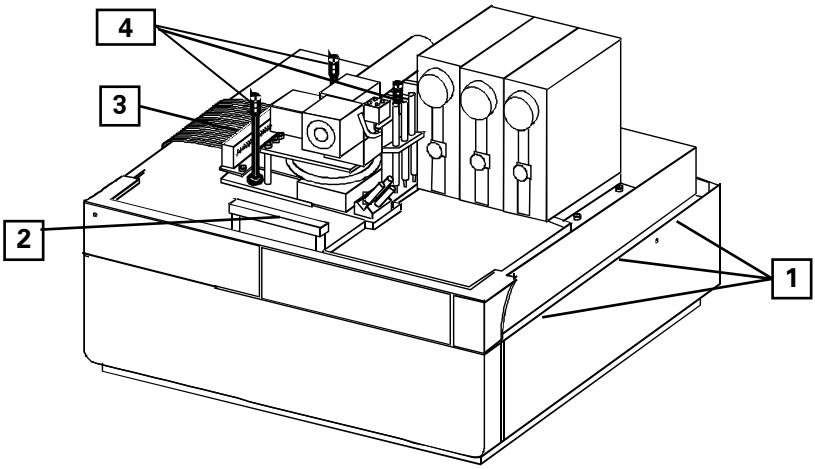
2 Finger nuts, 4 pieces

Fig. 4.6a Removing the light cover of the optical unit

5. Undo the six screws, three screws on each side of the instrument (Fig. 4.6b:1), fixing the measurement chamber to the chassis.
6. Lift the measurement chamber into the upper position from the handle (Fig. 4.6b:2). There are hinges in the rear and a gas spring (Fig. 4.6c:3) holds the chamber in the upper position.

Caution: The measurement chamber is very heavy. DO NOT leave your fingers between the measurement chamber and the bottom case.

7. Remove the three short finger nuts holding the cover (Fig. 4.6c:2). Then remove the cover plate (Fig. 4.6c:1) from the opposite position of the optical unit under the measurement chamber.



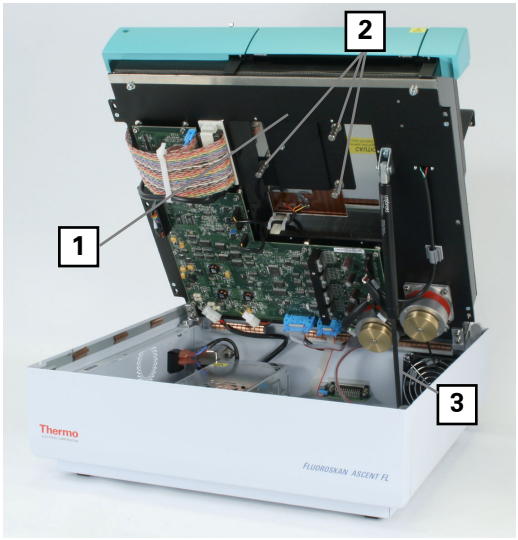
1 Six screws, three screws on each side of the instrument

2 Handle

3 Flat cable

4 Long finger nuts, 3 pieces

Fig. 4.6b Removing the optical unit



1 Cover plate

2 Short finger nuts, 3 pieces

3 Gas spring

Fig. 4.6c Cover plate

8. Lower the measurement chamber back into the down position from the handle.
9. Unplug the flat cable (Fig. 4.6b:3) and the lamp cable located beside the flat cable from the optical unit. DO NOT remove any other parts from the optical unit.
10. Undo the three long finger nuts (Fig. 4.6b:4) and remove the optical unit.
11. Lift the measurement chamber into the upper position from the handle.
12. Place the optical unit into the position where you removed the cover plate (Fig. 4.6c:1). Fix it with the long finger nuts and plug in the cable connectors, the flat cable and the lamp cable. No adjustments are needed.
13. Lower the measurement chamber back into the down position.
14. Place the cover plate instead of the removed optical unit and fix it with the short finger nuts.
15. Fit the six screws back to hold the measurement chamber and replace the instrument cover. The unfitted cover may increase stray light and harm the measurement.
16. To change the measurement direction, the optical unit and the cover plate are interchangeable.

4.7 Installing or removing the light shield

The light shield is only present in the Fluoroskan Ascent FL.

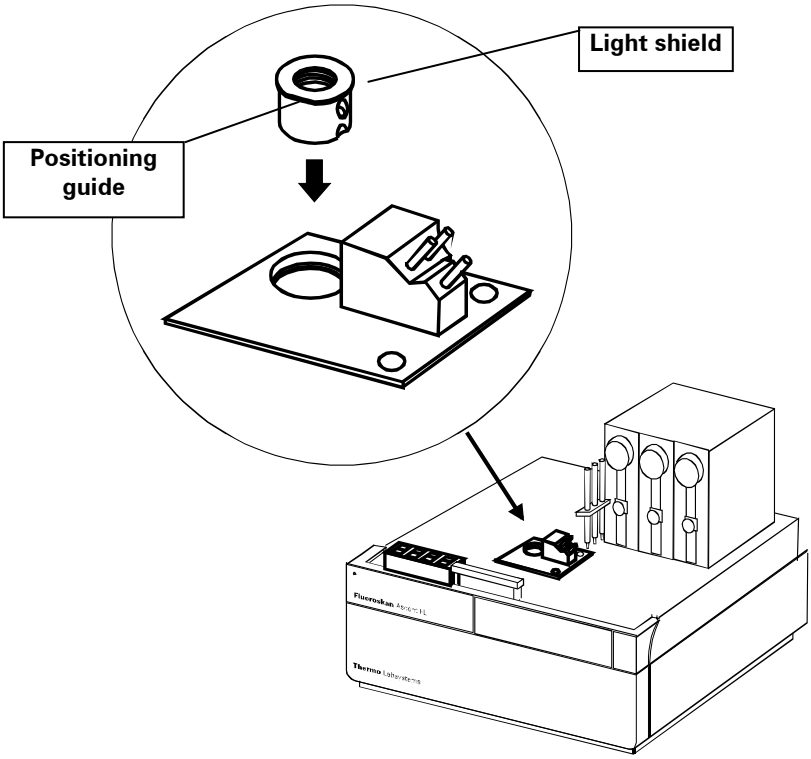


Fig. 4.7a Installing the light shield

1. Switch off the instrument and disconnect the mains power supply cable (Fig. 3.3:2).
2. Remove the instrument cover as described in Section 3.2 Releasing the transportation lock.
3. Undo the four finger nuts (Fig. 4.6a:2) and remove the light cover (Fig. 4.6a:1).
4. Undo the three long finger nuts (Fig. 4.6b:4) and remove the optical unit or, if the optical unit is below the measurement chamber, remove the cover plate (Fig. 4.6c:1).
5. When removing the light shield, use your little finger to lift the light shield up. When installing the light shield, ensure that the positioning guide is placed towards the front of the instrument against the corresponding guide in the holder (Fig. 4.7a and Fig. 4.7b). If the light

shield is not positioned correctly, the optical unit does not slot down into its correct place but stays swinging.

Caution: Mispositioning of the light shield will cause discrepancies in the measurement results or may cause the instrument not to work.

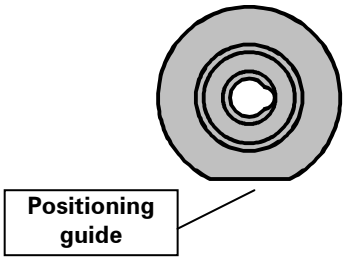


Fig. 4.7b Top view of the light shield

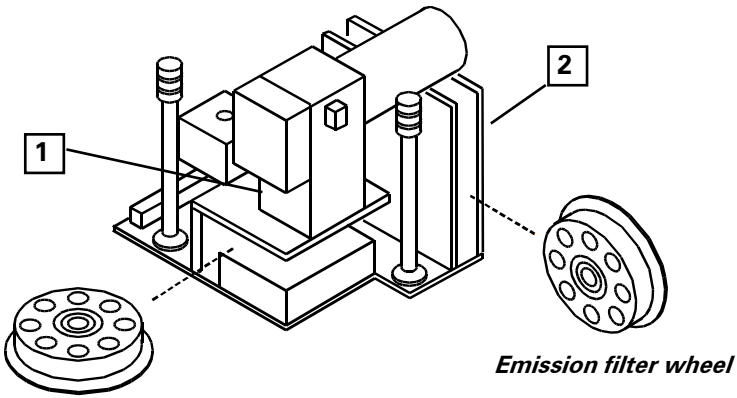
6. Fit the optical unit, light cover and the instrument cover back into their places.

4.8 Installing the filters

1. Switch off the instrument and disconnect the mains power supply cable (Fig. 3.3).
2. Remove the instrument covers as described in Section 3.2 Releasing the transportation lock points 1 – 4.
3. If the optical unit is above the measurement chamber, undo the four finger nuts (Fig. 4.6a:2) and remove the light cover (Fig. 4.6a:1). You can find more detailed instructions in Section 4.6 Changing the measurement direction.
4. If the optical unit is below the measurement chamber, undo the six screws (Fig. 4.6b:1) fixing the measurement chamber to the chassis. Lift the measurement chamber into the upper position. There are hinges in the rear and a gas spring (Fig. 4.6c:3) holds the chamber in the upper position.

Caution: The measurement chamber is very heavy. DO NOT leave your fingers between the measurement chamber and the bottom case.

5. Unplug the flat cable (Fig. 4.6b:3) and the lamp cable located beside the flat cable from the optical unit. Undo the three long finger nuts (Fig. 4.6b:4) and remove the optical unit and place it on a table.
6. The excitation filter wheel (Fig. 4.8a) can be removed by undoing the fitting screw (Fig. 4.8a:1). DO NOT touch the filter surfaces.
7. The emission filter wheel (Fig. 4.8a) can be removed by undoing the fitting screw (Fig. 4.8a:2). DO NOT touch the filter surfaces.
8. Select the filter wheel into which you want to install a filter. Normally the filters are factory installed in ascending wavelength order starting from position 1. Select the first free filter slot.



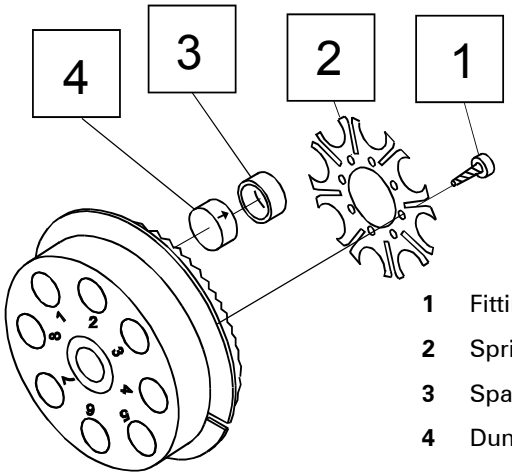
1 Fitting screw holding the excitation filter wheel in place

2 Fitting screw holding the emission filter wheel in place

Fig. 4.8a Filter wheels in the optical unit

9. Undo the fitting screws (Fig. 4.8b:1).

10. Remove the spring wheel (Fig. 4.8b:2).
11. Remove the spacer collar (Fig. 4.8b:3).
12. Remove the dummy filter or previously installed filter used (Fig. 4.8b:4). Install a new filter so that the small arrow on the filter rim points away from the filter wheel. The arrow shows the direction of the light flow.
13. Fit the spacer collar, spring wheel and the fitting screws. Fit the filter wheel back into place.



1 Fitting screw(s)

2 Spring wheel

3 Spacer collar

4 Dummy filter or previous filter used

Fig. 4.8b Filters in a filter wheel

14. Replace the optical unit, connect the flat cable and lamp cable. Lower the measurement chamber and replace the instrument cover.
15. Validate the changed filter pairs, refer to the instructions in Section 4.3.1 Validation and the Ascent Software User's Guide.

4.9 Shutdown

- Remove any microplates left on the plate carrier or breakable strips from the tip priming vessel. Dispose of all microplates and strips as biohazardous waste.
- Flush the pump(s) out thoroughly with distilled water. Empty all the tubings.
- Place the dispensing tips into the tip holder (Fig. 2.3:5).
- Switch off the Fluoroskan Ascent FL/Fluoroskan Ascent by pressing the power switch on the left-hand side of the instrument into the OFF position.
- Wipe the plate carrier surface and the external surfaces of the instrument with a soft cloth or tissue paper moistened with distilled water or a mild detergent solution.
- Push the plate carrier manually in.
- If you have spilt infectious agents on the plate carrier, disinfect with 70% pure ethanol in distilled water or some other disinfectant. See Section 8.2 Decontamination procedure.
- If you are not using the Fluoroskan Ascent FL/Fluoroskan Ascent for an extended period of time, always clean the external surfaces of the instrument.
- Finally put the dust cover on.

5. Maintenance

Note: Follow normal laboratory safety procedures with regard to biohazardous, infectious, radiologic or toxic materials when maintaining the instrument.

5.1 Routine cleaning of the instrument

For reliable operation keep the instrument free of dust and spills of liquids. We recommend you clean the case of the instrument periodically. A soft cloth dampened in mild detergent is sufficient. We recommend you service the instrument at least yearly.

If you believe liquid has entered the fluorometer, switch the instrument off and contact your local Thermo Fisher Scientific representative or Thermo Fisher Scientific Oy for technical service (see Sections 8.1 Service request protocol and 8.4 Shipping the instrument (or items). If any surfaces have been contaminated with biohazardous material, a sterilizing solution must be used.

The prescribed decontamination procedure (see Section 8.2 Decontamination procedure), or similar routine, must be performed before returning the instrument to the supplier for service or repair. All instruments must be accompanied by a completed and signed Certificate of Decontamination securely attached to the exterior of the packaging (see Section 8.3 Certificate of Decontamination).

5.2 Cleaning the optical system

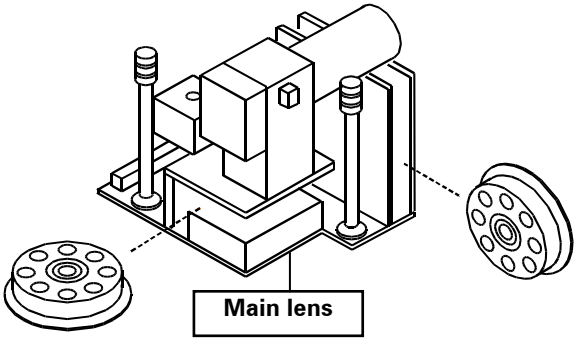


Fig. 5.2 Cleaning the optical unit

1. Switch off the instrument and disconnect the mains power supply cable. Locate the optical unit and remove it according to the instructions in Sections 3.2 Releasing the transportation lock and 4.6 Changing the measurement direction.
2. Clean the main lens (Fig. 5.2), the filters and the light shield (Fig. 4.7a) with a cloth dampened with 96% pure ethanol and afterwards with a lint-free cloth or a lens tissue.

DO NOT use any other liquids to clean the optical unit. Avoid any harsh treatment.

3. Replace all the removed parts and reconnect the instrument to the mains power supply.

5.2.1 Visual filter check

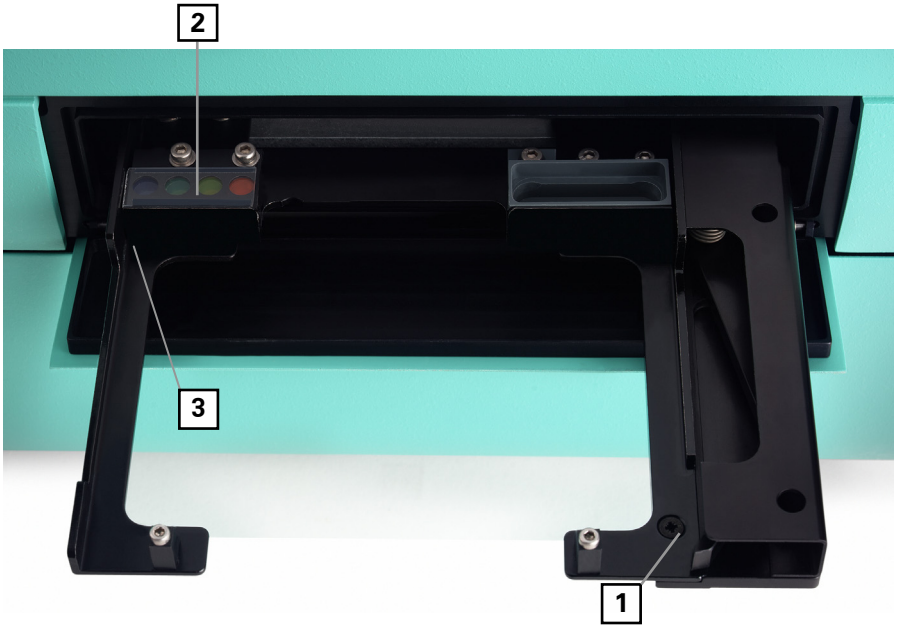
The useful life of a filter depends on environmental factors, such as dust, humidity and temperature. Filters have a one year warranty.

Carry out the visual check in the following way:

Visually check the filter(s) by holding it (them) against an even light source. If the color of the filter is even, then the filter is suitable for use. On the other hand, if the filter appears to be mottled or discolored, discard the filter since it is either damaged or defective.

The best alternative is to measure the filters with a spectrophotometer.

5.3 Cleaning the plate carrier



1 Plate carrier

2 Reference chip protection glass

3 A1 corner of the microplate

Fig. 5.3 Plate carrier

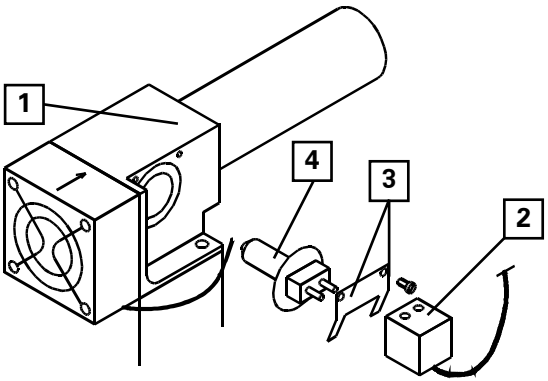
1. Clean the plate carrier (Fig. 5.3) with a cloth dampened with distilled water. In case of any spills of infectious agents, clean the plate carrier with disposable towels dampened with a disinfectant solution containing 2% glutaraldehyde. Always use disposable gloves. Place the disposable towels and gloves in a biohazardous waste container.
2. Clean the reference chip protection glass with a cloth dampened with 96% pure ethanol in distilled water and afterwards with a lint-free cloth or lens tissue.

5.4 Replacing the halogen lamp

1. Switch off the Fluoroskan Ascent FL/Fluoroskan Ascent and disconnect the mains power supply cable (Fig. 5.4:1). Remove the instrument cover according to the instructions in Section 4.6 Changing the measurement direction points 1 – 4. Locate the lamp (Fig. 5.5:4) on top of the optical unit (Fig. 5.5:1).
2. Open the two screws of the lamp connector (Fig. 5.5:2) and remove the connector.
3. Open the two screws of the lamp holder (Fig. 5.5:3) to release the lamp.
4. Replace the faulty lamp (Fig. 5.5:4) with the spare provided with the accessories. Contact your local Thermo Fisher Scientific representative if a spare lamp is required.

Caution: DO NOT touch the glass bulb with your bare fingertips. Note that the lamp is not symmetrical. The guide in the rim should point to the right.

5. Refit the lamp connector.
6. Replace both the light cover and the instrument cover.
7. Reconnect the instrument to the mains power supply.



1 Optical unit
2 Lamp connector
3 Lamp holder
4 Lamp

Fig. 5.4 Replacing the halogen lamp

5.5 Routine maintenance of optional dispensers and main lens

To obtain optimum performance and maximum useful life from the dispensers, it is important that the recommended cleaning maintenance instructions are followed.

Both fluorometry and luminometry are very sensitive detection technologies. Therefore, take special care to avoid any contamination of any parts of the dispenser tubings and follow all GLP (Good Laboratory Practice) recommendations.

5.5.1 Basic maintenance

1. The basic maintenance procedure should be performed regularly to ensure proper dispenser operation.
2. Rinse the dispenser tubings out thoroughly with distilled water after each use.
3. DO NOT allow the dispensers to run dry for more than a few cycles.
4. Inspect the dispensers for leaks and rectify any problems immediately.
5. Wipe up all spills on and around the dispensers immediately.

5.5.2 Extended maintenance

Clean the fluid path thoroughly using one of the procedures outlined below. There are three ways that the dispensers may be cleaned:

- Weak detergent
- 10% bleach
- Weak acid and base

5.5.2.1 Weak detergent or 10% bleach

Remove the dispensing heads from the dispensing positions and DO NOT let any cleaning fluids enter the measurement chamber. Use external containers.

1. Prime the dispensers with a weak detergent or 10% bleach solution and leave it in the dispensers with the syringes full for 30 minutes.
2. After the 30-minute period, remove the aspirate tubing from the detergent or bleach solution and remove all the fluid from the syringes and tubing into a waste container.

3. Flush the dispenser a minimum of 10 cycles with distilled water.

5.5.2.2 Weak acid and base in sequence

Remove the dispensing heads from the dispensing positions and DO NOT let any cleaning fluids enter the measurement chamber. Use external containers.

1. Prime the dispensers with 0.1 M NaOH and leave the solution in the dispensers for 10 minutes with the syringes full.

Note: 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 dispensers with distilled water.
3. Prime the dispensers with 0.1 M HCl and leave the solution in the dispensers for 10 minutes with the syringes full.
4. After the 10-minute period, remove the aspirate tubing from the 0.1 M HCl solution and remove all the fluid from the syringes and tubing into a waste container.
5. Flush the dispensers a minimum of 10 cycles with distilled water.

5.5.2.3 Cleaning the main lens

It is recommended that you check the main lens of the optical unit weekly.

1. Remove the optical unit if it is above the measurement chamber, or the cover plate if the optical unit is below the measurement chamber, as described in Section 4.6 Changing the measurement direction.
2. If needed, clean the main lens according to the instructions in Sections 5.2 Cleaning the optical system.

5.6 Periodic maintenance

There are three items which require periodic maintenance: tubing; syringe seals, and valves. If they become worn out, the symptoms are:

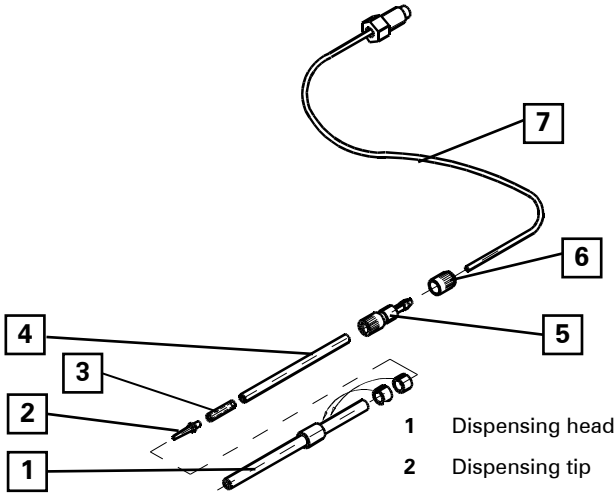
- Poor precision and accuracy
- A variable or moving air gap
- Leakage
- Drops and spills

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, the easiest and most economical way is to replace one component at a time in the following order: (1) dispensing or aspirate tubings and/or dispensing tip, and (2) syringe.

5.6.1 Replacing the dispenser tubings

1. To remove either the dispensing tube or the aspirate tube assembly from the valve, gently loosen the fittings either manually or using a 7.9 mm (5/16 in.) wrench. Unscrew the fittings and remove the tubing.
2. We recommend you replace the complete assemblies always when replacement is necessary. Alternatively, only some parts of the assemblies can also be replaced. If only the dispensing tubing is changed, first remove the complete dispensing tube assembly from the dispenser unit.

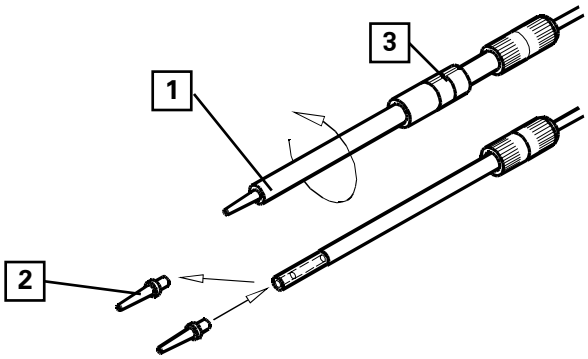


1 Dispensing head tube
2 Dispensing tip
3 Silicone tubing
4 Internal dispenser head tube
5 Brass tube holder
6 Brass locking ring
7 Dispensing tubing

Fig. 5.6.1 Structure of the complete dispensing tube assembly

3. Remove the dispensing head tube (Fig. 5.6.1:1) from the brass tube holder (Fig. 5.6.1:5).
4. Loosen the brass locking ring (Fig. 5.6.1:6).
5. Remove the dispensing tip (Fig. 5.6.1:2) and the connecting piece of silicone tubing (Fig. 5.6.1:3).
6. Remove the internal dispenser head tube (Fig. 5.6.1:4), the brass tube holder and the brass locking ring.
7. Insert the new dispensing tubing into the brass tube holder/locking ring and the internal dispenser head tube.
8. Connect the dispensing tip with the connecting piece of silicone tubing into the new dispensing tubing.
9. Fit the dispensing tip and tubing into the dispenser head tube. When you push the dispensing tubing gently towards the tip, the internal dispenser head tube should be visible about 1 mm. This can be adjusted through the length of the connecting piece of silicone tubing.
10. First screw the brass tube holder onto the dispenser head tube. Gently tighten the tubing and ensure that the tube end stays in the silicone tubing. Then tighten the brass locking ring.
11. To fit a new tubing, insert the fitting into the valve and tighten it finger-tight. Using a 7.9 mm (5/16 in.) wrench, turn the fitting another quarter to half turn.

5.6.2 Replacing the dispensing tip



1 Dispensing head tube

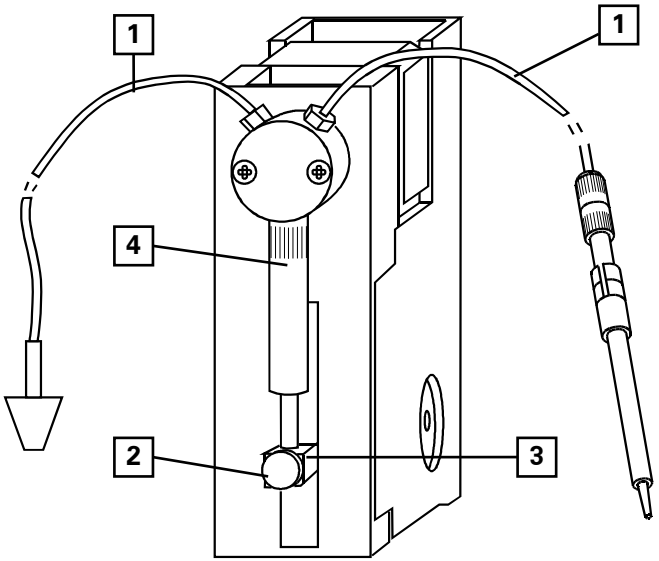
2 Dispensing tip

3 Brass tube holder

Fig. 5.6.2 Replacing the dispensing tip

1. Remove the dispensing head tube (Fig. 5.6.2:1) from the brass tube holder (Fig. 5.6.2:3).
2. Replace the dispensing tip (Fig. 5.6.2:2) connected with a small piece of silicone tubing in the dispensing tube.
3. Replace the dispensing head tube.

5.6.3 Replacing the dispenser syringe



1 Aspirate and complete dispensing tube assemblies
2 Plunger lock screw
3 Plunger holder arm
4 Dispenser syringe (1.0 ml) and plunger

Fig. 5.6.3 Replacing the dispenser syringe

1. Remove the liquid from the dispenser syringe (Fig. 5.6.3:4) and from the tubings.
2. Switch off the power from the instrument.
3. Push the plunger manually into the upper position.
4. Loosen the plunger lock screw (Fig. 5.6.3:2) approximately three full turns.
5. Pull the plunger holder arm (Fig. 5.6.3:3) firmly down.
6. Unscrew the syringe from the valve.
7. To fit the new dispenser syringe, screw the syringe into the valve, pull the syringe plunger down to the plunger holder arm and screw it into place. Make sure the plunger lock screw is securely tightened.

6. Troubleshooting Guide

Note: DO NOT use the instrument if it appears that it does not function properly.

6.1 Troubleshooting

Problem	***Cause***	***Action***
Instrument does not turn on correctly	No power connection	Check the power cable
No connection between instrument and PC	Incorrect software type	Check that the software is installed for the correct instrument model
	Loose serial cable connections	Check the cable connections
	Software in simulation mode	Switch the connection to the instrument
Too high background	No liquid in the well	Always use some liquid in the blank wells
	Unclean plate	Use a disposable plate only once
	Contaminated reagents	Replace the reagents used
	Microplate material	Check if the plate manufacturer or material has changed
	Unsuitable filter pair	Ensure that the filters used are suitable for the fluorochrome
	Contaminated tubing	Replace/clean the tubing
	Phosphorescence from the plastic	Use only plastics designed for fluorometry/luminometry without phosphorescence

Continued

Problem	***Cause***	***Action*** *Cont.*
Too low/high signal	Changed scaling factor	Check the scaling factor used
	Microplate material	Check if the plate manufacturer or material has changed
	Unsuitable filter pair	Ensure that the correct filter pair is used
	Unsuitable filter pair	Ensure that the filters used are suitable for the fluorochrome
Even, low signal level for the whole plate	Bottom reading of non-transparent plate	Use top reading
	Bottom reading when drop plate in its place	Remove the drop plate
Too large deviation between replicates	Unclean plate	Use a disposable plate only once
	Dust or dirt in the wells	Keep the plates protected before use
	Foaming in the sample	Use a lower dispensing or shaking speed
		Use correct pipetting techniques (reverse)
Illogical results	Plate inserted with incorrect orientation	Rotate the plate with the A1 well facing towards the upper left corner
Too low or variable volume from dispenser	Inadequate priming	Reprime the dispenser
		Use tip priming during the session
	Incorrect dispensing height	Check that the dispenser heads are positioned at the correct height

Continued

Problem	***Cause***	***Action*** *Cont.*
Too low or variable volume from dispenser	Incorrect dispensing height	Check that the dispenser head positions in the instrument and in the software settings are identical
		Use plate adapters with low 384-well plates
	Loose syringe, plunger or tubings	Check all the connections in the dispenser
	Aspiration tube not in the liquid	Ensure that there is a sufficient volume of liquid in the vial
		Ensure that the aspiration tube end weight is at the bottom of the vial and attach it firmly, if necessary
Liquid droplets outside the wells after dispensing	Incorrect plate template used	Check the plate type used in the software
	Too high total volume used	Use smaller volumes
	Lid used with the plate	DO NOT use plate lids with the dispensers
	Damaged dispensing tip	Replace the tip
	Incorrect installation of the dispensing head	Check that the dispensing head is inserted deep enough
	Incorrectly adjusted dispenser head assembly	Contact authorized technical service
Noise from the instrument	Light shield not removed when using high plates	Remove the light shield (FL only)

Continued

Problem	***Cause***	***Action*** *Cont.*
Noise from the instrument	Lid left on the plate	Remove the light shield or the lid
	Dispenser head installed to the incorrect height	Check the dispenser/plate height
	Filter wheel too tight	Loosen the filter wheel screw
	Transportation lock not removed	Remove the transportation lock
	Plate adapter for low 384-well plates left under a 96-well plate	Remove the adapter when using 96-well plates
Plate carrier moves incorrectly	Foreign object, e.g., a tip priming strip, obstructs the plate carrier movement	Ensure that there are no loose objects inside the instrument
"Too high background drift" message	Dirty measurement window or reference chip protection glass	Clean the measurement window and the reference chip protection glass
Accidental dispensing into the instrument		Contact authorized technical service

6.2 Error messages

Error messages issued by the instrument are listed in the Reference part of the Ascent Software User's Guide in the chapter on warning and error messages.

7. Frequently asked questions (FAQ) about the Fluoroskan Ascent FL and Fluoroskan Ascent

Q: Can Fluoroskan Ascent be upgraded for luminometric applications?

A: It is not possible to add the luminometric option to the Fluoroskan Ascent. Only the Fluoroskan Ascent FL can measure both fluorescence and luminescence.

Q: Is the wavelength marked on each Fluoroskan Ascent FL and Fluoroskan Ascent filter?

A: Wavelength as well as date information is printed on each Fluoroskan filter frame. On some filters the half-bandwidth (HBW) is also printed.

Q: Are Petri dish adapters standard accessories that come with the Fluoroskan Ascent FL and Fluoroskan Ascent?

A: Petri dish adapters are not standard accessories that come with the reader and thus they should be ordered separately.

Q: When is the adapter for low 384-well plates needed?

A: The adapter for low 384-well plates is needed when you dispense into low 384-well plates (Fluoroskan Ascent FL and Fluoroskan Ascent). If the plate height is less than 13.5 mm, an adapter must be used to raise the plate. In luminometry it is also needed for the measurement of low 384-well plates (Fluoroskan Ascent FL).

Q: What is the wide wavelength range option for the Fluoroskan Ascent FL?

A: The Fluoroskan Ascent FL photomultiplier tube (PMT) provides good sensitivity in both fluorometry and luminometry. With this PMT the emission wavelength range for the Fluoroskan Ascent FL ranges from 360 to 670 nm. If the "Wide Wavelength Range Option" is selected, the PMT of the Fluoroskan Ascent is installed into the FL. The emission wavelength range which can then be measured, ranges from 360 to 800 nm. However, the luminometric sensitivity is decreased about one decade. The fluorometric sensitivity is the same with both types of PMTs.

Q: **How do the fluorescence reference chips work, which are installed into the Fluoroskan Ascent FL and Fluoroskan Ascent plate carrier?**

A: The fluorescent chips are installed into the unit to keep the instrument signal level stable. When a filter pair is validated, the instrument measures all the four chips in the plate carrier and then automatically selects the most suitable chip. The chip measurement value is stored in the instrument memory. When the measurement is started, autocalibration is performed. The instrument measures this chip during the autocalibration process and compares it with the value in memory. The correction factor is calculated and all further measurement values are multiplied by this factor.

Q: **How is the scaling factor in Ascent Software used?**

A: The scaling factor is a constant that multiplies all the readings from the instrument. It is defined separately for each filter pair and beam size (fluorometry) or filter position (luminometry). The scaling factor can be used to convert the instrument reading to a desired level or to adjust several instruments to give similar signal values.

Q: **What plate colors can be used for fluorescence?**

A: Best sensitivity 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 plates can be used, but the sensitivity is slightly lower and the difference is dependent on the filter pair used. When you carry out bottom reading, only transparent or transparent bottomed plates can be used.

Q: **When can I use white plates in fluorometry?**

A: In assays such as DNA quantitation with PicoGreen, white plates can be used as the obtained fluorescent signal level is not very high. The sensitivity is also slightly better when using white plates. White plates can also be used in, for example, GFP quantitation; however, white plates should not be used when the signal level is very high.

Q: What plate colors can be used for luminescence?

A: White plates are generally used for luminometric measurements to enhance the signal and to avoid possible crosstalk from high signals in neighboring wells.

Q: Can dispensing be performed into 864-well plates?

A: No, dispensing into 864-well plates is not possible.

Q: **Can the light shield be removed from the Fluoroskan Ascent FL while carrying out fluorometric measurements?**

A: Yes, as the light shield is only needed with luminometric measurements.

Q: **When is the light shield needed with the Fluoroskan Ascent FL?**

A: The light shield is needed in luminometric measurements and does not interfere with fluorometric measurements. The light shield should be used with 96- and 384-well plates. If the plates are higher than 15 mm, the light shield must be removed.

Q: **How many filters can be installed into the Fluoroskan Ascent FL and Fluoroskan Ascent?**

A: Both excitation and emission filter wheels in the Fluoroskan Ascent can hold a maximum of eight filters. The Fluoroskan Ascent FL excitation filter wheel can hold a maximum of eight filters and the emission wheel a maximum of six filters.

Q: **What plate types can be measured with the Fluoroskan Ascent?**

A: The Fluoroskan Ascent FL and Fluoroskan Ascent can measure plate formats from 1 to 864. Customized plates with maximum dimensions of 90 mm x 143 mm x 25 mm can be used.

Q: **Does the Fluoroskan Ascent have different beams for different plate types?**

A: The Fluoroskan Ascent FL and Fluoroskan Ascent have two beam diameter settings. The normal beam with a diameter of 3 mm is generally recommended for 96-well plates and larger wells. The small beam with a diameter of 1.5 mm is recommended for 384-well plates.

Q: **Is it possible to measure 96-well PCR plates with the Fluoroskan Ascent FL and Fluoroskan Ascent?**

A: Yes, and you can use the Fluoroskan Ascent FL and Fluoroskan Ascent for measuring both PCR plates and 0.2 ml PCR tubes but you need a special adapter. This adapter is suitable for, for example, Hybaid Omniplates, Robbins Cycloplates and Axygen PCR microplates or tubes from Greiner or Sarstedt.

Q: **Do I have to make any modifications to the instrument if I want to measure fluorescence and luminescence from the same well?**

A: No, you must only select separate measurement steps in the software, which means that you can use both measurement technologies in one session for measuring the same wells.

Q: **What kind of plates are best to use with the Fluoroskan Ascent FL (luminometric measurements) when you have to reduce the crosstalk as much as possible and minimize the background?**

A: There are clearly two options. You should use either white Microlite 1+ plates (Cat. no. 7571) or white universal binding plates (Cat. no. 9502887).

Q: **What kind of plate adapters are available for the Fluoroskan Ascent FL and Fluoroskan Ascent?**

A: The adapters listed below are available. Other adapters are available on request.

5210310	Adapter for Petri dish 2 x ø 59 mm
5210300	Adapter for Costar Petri dish ø 95 mm
5210380	Adapter for Falcon/Greiner Petri dish ø 93 mm
5210330	Adapter for Petri dish 6 x ø 40 mm
5210320	Adapter for low 384-well plates
5210390	Robotic 384-adapter
5210340	Adapter for Terasaki plates
5210630	Adapter for PCR tubes and 96-well PCR plates

8. Instrument Service

8.1 Service request protocol

If the Fluoroskan Ascent FL or Fluoroskan Ascent 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.

The Thermo Fisher Scientific representative or distributor takes care of sending the Thermo Fisher Scientific technical service department a Feedback Form (i.e., the Complaint-Order), which 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 Feedback Form to the technical service department.

Check Section 8.4 Shipping the instrument (or items). You will find instructions on how to proceed before shipping the instrument for service.

Check that any necessary decontamination procedure has been carried out before packing. See Sections 8.2 Decontamination procedure and 8.3 Certificate on Decontamination. Ensure that the Certificate of Decontamination is 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.

8.2 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 recommended when infectious substances have been in direct contact with any part(s) of the instrument.

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

We strongly recommend that the complete decontamination procedure is performed before relocating the instrument from one laboratory to another.

Example of disinfectants

- Formaldehyde solution 10%
- Pure ethanol 70% (in distilled water)
- Virkon solution 1 – 3%
- Glutaraldehyde solution 4%
- Chloramine T

Caution: Always use disposable gloves and protective clothing and operate in a well-ventilated area.

1. Prepare the disinfectant: 200 ml 10% formaldehyde solution or 200 ml 4% glutaraldehyde solution (or another agent recommended by your safety officer).
2. Empty the plate carrier.
3. Switch off the power and disconnect the mains power supply cable and the computer cable.
4. Disinfect the outside of the instrument using a cloth dampened with 70% pure ethanol in distilled water.
5. Place the instrument in a large plastic bag. Ensure that all the lids are open.
6. Place a cloth soaked in the prepared solution into the bag. Ensure that the cloth does not make 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% pure ethanol in distilled water.

11. After performing this decontamination procedure, label the instrument with a signed and dated Certificate of Decontamination.

8.3 Certificate of decontamination

The decontamination procedure is required prior to 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. See Section 8.2 Decontamination procedure.

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 items, ensure that they are fully decontaminated. Confirm A or B status (see below).

Certificate of Decontamination

Name: _____

Address: _____

Tel./Fax: _____

Instrument: _____ 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: _____
_____
_____

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): _____

8.4 Shipping the instrument (or items)

When you ship the instrument for service remember to:

1. Empty the dispenser (s) and remove the tube assembly.
2. Remove any loose items from the plate carrier, for example, adapters, plates and priming vessels.
3. Remove the power cable as well as the serial cable.
4. Decontaminate the instrument beforehand.
5. Fit the transportation lock.
6. Pack the instrument according to the enclosed packing instructions.
7. Use the original packaging to ensure that no damage will occur to the instrument during shipping. Any damage will incur additional labor charges.
8. Inform about the use of hazardous materials.
9. Enclose a dated and signed Certificate of Decontamination (see Section 8.3 Certificate of Decontamination) both inside and attached to the outside of the box in which you will return your instrument or other items.
10. Indicate the fault after you have been in touch with your local Thermo Fisher Scientific representative or the Thermo Fisher Scientific technical service department.
11. Enclose the return authorization number (RGA) given by the Thermo Fisher Scientific representative.

See Section 14.1 General specifications for details on storage and transportation temperatures.

8.5 Service contracts

We strongly recommend that you maintain and service the instrument every twelve 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 authorized technical service for further details.

9. Disposal of the Instrument and Materials

9.1 Disposal of the instrument

For the disposal of the instrument do the following:

- Decontaminate the instrument prior to disposal. See Sections 8.2 Decontamination procedure and 8.3 Certificate of Decontamination.
- 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.

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 further information, contact your local Thermo Fisher Scientific representative.

9.2 Disposal of materials

Refer to local regulations for the disposal of infectious material.

The samples can be potentially infectious. Dispose of all used microplates, PCR tubes, disposable gloves, syringes, disposable tips, etc. as biohazardous waste.

10. Ordering Information

10.1 Product code numbers

Microplate Fluorometers

Cat. no.	*Instrument*
5210470	Fluoroskan Ascent
5210480	Fluoroskan Ascent with dispenser
5210450	Fluoroskan Ascent FL
5210460	Fluoroskan Ascent FL with dispenser

Fluorometric Filters

Cat. no.	*Item*	*HBW*
1423202	Excitation filter 320 nm	40 nm
1423552	Excitation filter 355 nm	38 nm
1423902	Excitation filter 390 nm	20 nm
142430210	Excitation filter 430 nm	10 nm
142440210	Excitation filter 440 nm *	10 nm
1424442	Excitation filter 444 nm *	12 nm
1424852	Excitation filter 485 nm *	14 nm
1425302	Excitation filter 530 nm	10 nm
1425442	Excitation filter 544 nm	15 nm
142578206	Excitation filter 578 nm	7 nm
1425842	Excitation filter 584 nm	16 nm
1426462	Excitation filter 646 nm	10 nm

***** Specified to be used as either excitation or emission filter

Fluorometric Filters Cont.

Cat. no.	*Item*	*HBW*
1424052	Emission filter 405 nm	50 nm
1424602	Emission filter 460 nm *	24 nm
1424852	Emission filter 485 nm *	14 nm
1425102	Emission filter 510 nm *	10 nm
1425182	Emission filter 518 nm	12 nm
1425202	Emission filter 520 nm *	10 nm
1425272	Emission filter 527 nm	15 nm
1425382	Emission filter 538 nm	25 nm
1425552	Emission filter 555 nm	25 nm
1425902	Emission filter 590 nm	14 nm
1426042	Emission filter 604 nm	10 nm
1426122	Emission filter 612 nm	6 nm
1426202	Emission filter 620 nm *	10 nm
142678210	Emission filter 678 nm	10 nm
1426802	Emission filter 680 nm	20 nm

***** Specified to be used as either excitation or emission filter

Other wavelengths available on request, see Section 10.3 Ordering filters.

Upgrade Kits for Fluoroskan Ascent

Item	*Cat. no.*
1st Dispenser Kit	2805620
Additional Dispenser Kit	2805630

Upgrade Kits for Fluoroskan Ascent FL

Item	*Cat. no.*
1st Dispenser Kit	2805621
Additional Dispenser Kit	2805630
Wide Wavelength Range Kit (PMT)	2805760

Accessories

Item	*Cat. no.*
Adapter for Costar Petri dish ∅ 95 mm	5210300
Adapter for Falcon/Greiner Petri dish ∅ 93 mm	5210380
Adapter for Petri dish 2 x ∅ 59 mm	5210310
Adapter for low 384-well plates	5210320
Robotic 384-adapter	5210390
Adapter for Petri dish 6 x ∅ 40 mm	5210330
Adapter for Terasaki plate	5210340
Adapter for PCR tubes and 96-well PCR plates	5210630
Drop plate	2805880
Halogen lamp	1410130
Light shield	1006470
Dispensing tip	1047661
Complete dispensing tube assembly (Fig. 3.4:2 and Fig. 5.7.1)	24071580
Dispensing tubing (Fig. 5.7.1:7)	24071500
Dispensing tip (Fig. 5.7.1:2) (10 pieces min. order)	1047661
Aspirate tube assembly (Fig. 3.4:1), incl. tubing and end weight	2805690
Dispenser syringe (1.0 ml) and plunger (Fig. 3.4:4)	24071490
Syringe seal	2805600
3-port valve	2805610
Leakage tray (Fig. 2.3:4)	10478030
Red adjustment collar (Fig. 4.5.4b:2)	1002820
RS-232C interface cable D9 Female/D25 Female	2305290
Dust cover	1610460
Fluoroskan Ascent and FL upgrade	2805660CD

Thermo Scientific Microtiter Plates

Cat. no.	*Description*	*Bottom*	*Qty*
Microtiter Plates for Immunoassay			
3355	Immulon 1B Plate	Flat	50/box
3555	Immulon 1B Plate	U	50/box
3455	Immulon 2 HB Plate	Flat	50/box
3655	Immulon 2 HB Plate	U	50/box
3855	Immulon 4 HBX Plate	Flat	50/box
9502227	96 Well Plate UB	Flat	50/box
95029330	96 Well Plate EB	Flat	50/box
95029780	96 Well Plate Sterile	Flat	40/box

Cat. no.	*Description*	*Bottom*	*Qty*
Microtiter Plates for Immunoassay, Strips and Assemblies			
6310	Immulon 1 B 1x12 Strip assembled	Flat	100/box
6301	Immulon 1 B 1x12 Strip	Flat	320/box
6381	Immulon 1 B 1x8 Strip assembled	C	100/box
6505	Immulon 1 B 2x8 Strip assembled	Flat	100/box
6521	Immulon 1 B 2x8 Strip assembled	C	100/box
6309	Immulon 2 HB 1x12 Strip assembled	Flat	100/box
6302	Immulon 2 HB 1x12 Strip	Flat	320/box
6382	Immulon 2 HB 1x8 Strip assembled	C	100/box
6506	Immulon 2 HB 2x8 Strip assembled	Flat	100/box
6522	Immulon 2 HB 2x8 Strip assembled	C	100/box
6405	Immulon 4 HBX 1x12 Strip assembled	Flat	100/box
6404	Immulon 4 HBX 1x12 Strip	Flat	320/box
6484	Immulon 4 HBX 1x8 Strip assembled	C	100/box
6508	Immulon 4 HBX 2x8 Strip assembled	Flat	100/box
6524	Immulon 4 HBX 2x8 Strip assembled	C	100/box
95029350	Strip 1x8 assembled UB	Flat	50/box
95029100	Strip 1x8 assembled EB	Flat	50/box
95029440	Strip 1x8 assembled UB	Round	50/box
95029370	Strip 1x12 assembled UB	Flat	50/box
95029140	Strip 1x12 assembled EB	Flat	50/box
95029390	Breakable Strip 1x8 Assembled UB	Flat	50/box
95029180	Breakable Strip 1x8 Assembled EB	Flat	50/box
95029430	Breakable Strip 1x8 Assembled EB	Round	50/box

Thermo Scientific Microtiter Plates Cont.

Cat. no.	*Description*	*Bottom*	*Qty*
Microtiter Plates for Luminescence			
7416	Microlite 1 Plate	Flat	50/box
7417	Microlite 2 Plate	Flat	50/box
7418	Microlite TCT Plate, sterile	Flat	50/box
7571	Microlite 1+ Plate	Flat	50/box
7572	Microlite 2+ Plate	Flat	50/box
7521	Microlite 1+ Plate	U	50/box
7522	Microlite 2+ Plate	U	50/box
9502887	White 96 Well Plate UB	RE	50/box
95029580	White 96 Well Plate EB	RE	50/box
95029770	White 96 Well Plate Sterile	RE	40/box

Cat. no.	*Description*	*Bottom*	*Qty*
Microtiter Plates for Luminescence, Strips and Assemblies			
7421	Microlite 1 1x12 Strip assembled	Flat	100/box
7403	Microlite 1 1x12 Strip	Flat	320/box
7400	Microlite 2 1x12 Strip assembled	Flat	100/box
7410	Microlite 2 1x12 Strip	Flat	320/box
7561	Microlite 1+ 1x12 Strip assembled	Flat	100/box
7566	Microlite 1+ 1x12 Strip	Flat	320/box
7562	Microlite 2+ 1x12 Strip assembled	Flat	100/box
7567	Microlite 2+ 1x12 Strip	Flat	320/box
95029510	White Strip 1x8 assembled UB	Flat	50/box
95029530	White Strip 1x12 assembled UB	Flat	50/box
95029660	White Breakable Strip 1x8 Assembled UB	Flat	50/box

Thermo Scientific Microtiter Plates Cont.

Cat. no.	*Description*	*Bottom*	*Qty*
Microtiter Plates for Fluorescence			
7605	Microfluor 1 Black Plate	Flat	50/box
7005	Microfluor 1 Black Plate	U	50/box
7805	Microfluor 2 Black Plate	Flat	50/box
7205	Microfluor 2 Black Plate	U	50/box
7705	Microfluor 1 White Plate	Flat	50/box
6905	Microfluor 1 White Plate	U	50/box
7905	Microfluor 2 White Plate	Flat	50/box
7105	Microfluor 2 White Plate	U	50/box
9502867	Black 96 Well Plate UB	Flat	50/box
95029120	Black 96 Well Plate EB	Flat	50/box
95029840	Black 96 Well Plate Sterile	Flat	40/box

Cat. no.	*Description*	*Bottom*	*Qty*
Microtiter Plates for Fluorescence, Strips and Assemblies			
95029450	Black Strip 1x8 assembled UB	Flat	50/box
95029490	Black Strip 1x8 assembled EB	Flat	50/box
95029470	Black Strip 1x12 assembled UB	Flat	50/box
95029500	Black Strip 1x12 assembled EB	Flat	50/box
95029800	Black Breakable Strip 1x8 Assembled UB	Flat	50/box

Cat. no.	*Description*	*Qty*
Microtiter 384 Well Plates		
95040000	384 Round Well Plate	50/box
95040010	White 384 Round Well Plate	50/box
95040020	Black 384 Round Well Plate	50/box
95040130	384 Round Well Plate Sterile with lid	40/box
95040230	White 384 Round Well Plate Sterile with lid	40/box
95040330	Black 384 Round Well Plate Sterile with lid	40/box

Thermo Scientific Microtiter Plates Cont.

Cat. no.	*Description*	*Bottom*	*Qty*
Microtiter Streptavidin-Coated Plates			
95029263	BioBind Strip 1x8 Assembled	Flat	5/box
95029293	BioBind Breakable Strip 1x8 assembled	Flat	5/box
95029273	White BioBind Strip 1x8 Assembled	Flat	5/box
95029303	White BioBind Br. Strip 1x8 Assembled	Flat	5/box
95029283	Black BioBind Strip 1x8 Assembled	Flat	5/box

Cat. no.	*Description*	*Qty*
Microtiter Plate Accessories		
5500 [1)]	Universal Polystyrene Lid, Sterile	100/box
5550 [1)]	Styrene Individually Wrapped Lid, Sterile	50/box
9503210 [2)]	Microplate Lid, 96-well	15/box
9503220 [2)]	Microplate Lid	15/box
6305	Vinyl Lid for 1x12 strip assembly	100/box
6604 [1)]	Holder for 1x12 strip assembly	10/box
6000 [1)]	Workstation 1x12 strip assembly	1/box

[1)] Accessories for ex-Dynex products

[2)] Accessories for ex-Thermo Scientific products

10.2 List of recommended spare parts

Cat. no.	*Item*	*1–2 unit(s)/year*	*10 units/year*
1410130	Halogen lamp 12V/30W	1	10
24071580	Complete dispensing tube assembly	1	5
24071500	Dispensing tubing	2	8
1047661	Dispensing tip	4	20
2805690	Aspirate tube assembly	1	5
24071490	Dispenser syringe (1.0 ml) and plunger	1	5
1006470	Light shield	1	3
1002820	Red adjustment collar	2	10

10.3 Ordering filters

Filters play an essential role in fluorometric measurements. When ordering special filters, the better information you provide, the better the measurement results will be.

A special filter order should include the following details:

1. Name of the application
2. Excitation and emission spectra
3. Peak or center wavelengths of the excitation and emission spectra
4. Reference article

The peak or center wavelengths of both filters are not enough for optimizing the filters. If you only want to order an excitation or emission filter, you also have to tell which filter is used as the pair.

The excitation and emission spectra are the best and easiest way to design the best possible filter pair for the user. If you have a reference article in addition to the name of your application, the order will be perfect.

Enclosed you can find the filter ordering form (see Appendix B).

Fluorescent materials absorb light energy of some characteristic wavelength band, undergo an atomic change, and instantaneously emit light of some longer wavelength band. The most common fluorescent materials have well-characterized excitation and emission spectra. Fig. 10.3a shows the excitation and emission spectra for a fluorescent material. The excitation and emission bands are each fairly broad with a half-bandwidth of approximately 40 nm, and the wavelength difference (Stokes shift) between the excitation and emission maximums is small, just 32 nm.

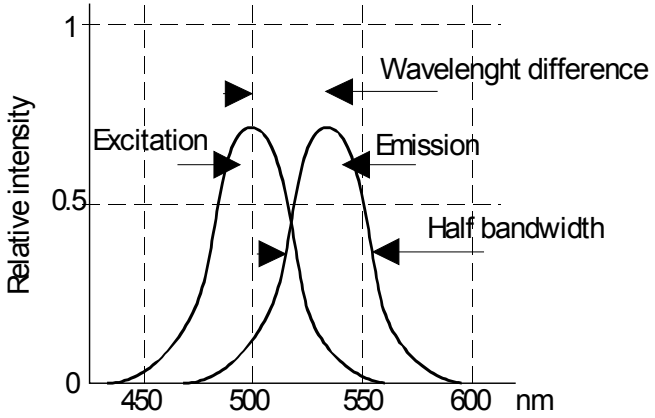


Fig. 10.3a Excitation and emission spectra

The requirement for any fluorometric system is to excite the fluorescent material with monochromatic light and permit observation of the subsequent emission. Because the intensity of stray, non-absorbed, excitated light is usually many tens of thousands of times greater than that of the emission light, some type of spectral separation is necessary to produce the contrast required for observation. Fig. 10.3b shows the excitation and emission spectra for an optimized pair of excitation and emission filters.

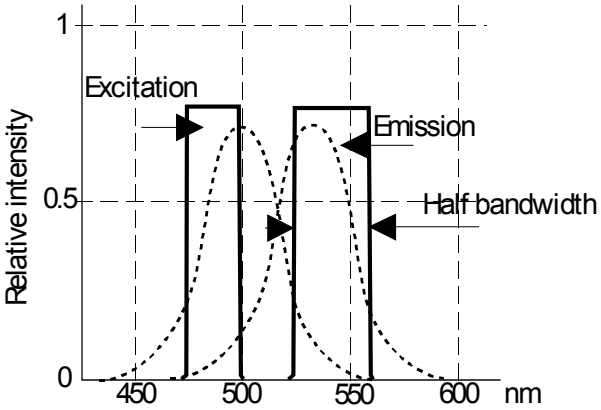


Fig. 10.3b An optimized pair of excitation and emission filters

In Fig. 10.3c the same spectral situation is shown, but transmission is on a logarithmic scale. Each filter deeply blocks the other, providing a pure emission signal.

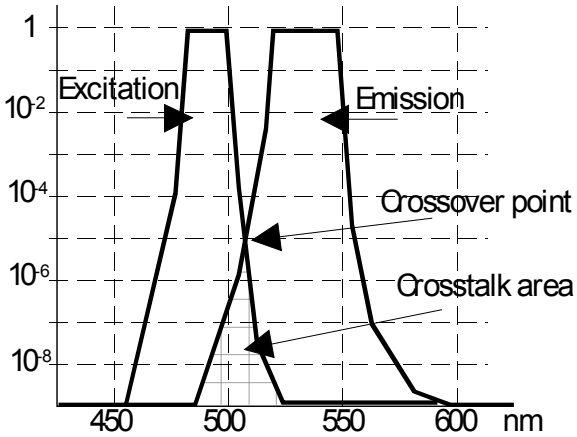


Fig. 10.3c Excitation and emission spectra on a logarithmic scale

This example shows that the center wavelengths of the filters are not necessarily the same as those of the fluorescent material's excitation and emission spectra. In this way the final result is better. If the crossover point is located too high, the background signal is high and the measurement sensitivity poor.

10.4 Fluorochromes and corresponding filter pairs

Fluorochrome	*Ex/Em*	*Filters (Cat. no.)*	*Notes*
Acridine Orange	485/538	1424852/1425382	+ DNA
alamarBlue	544/590	1425442/1425902	
AlexaFluor 488	485/518 or 538	1424852/1425182 or 1425382	
6-Aminoquinoline	355/544	1423552/1425442	
AFC	390/510	1423902/1425102	
AttoPhos	444/555	1424442/1425552	
Calcein	485/538 or 518	1424852/1425382 or 1425182	
Chlorophyll	444/680	1424442/1426802	
Cy3	544/590	1425442/1425902	
Cy5	646/678	1426462/142678210	
DAPI	355/460	1423552/1424602	+ DNA
EGFP	485/520 or /527	1424852/1425202 or 1425272	
EYFP	485/527	1424852/1425272	
FAM	485/518	1424852/1425182	
FITC	485/538 or 518 or 527	1424852/1425382 or 1425182 or 1425272	
Fluo 3	485/538 or 527	1424852/1425382 or 1425272	
Fluo 4	485/518	1424852/1425182	
Fluorescein	485/538 or 518 or 527	1424852/1425382 or 1425182 or 1425272	

Ex = Excitation filter; Em = Emission filter *Continued*

Cont.

Fluorochrome	***Ex/Em***	***Filters (Cat. no.)***	***Notes***
Fluorescein diacetate	485/538	1424852/1425382	
Fura 2	340 and 380/510	1423402 and 1423802/1425102	Only slow Ca^{+2} flux
Hoechst dyes 33258 and 33348	355/460	1423552/1424602	
HPPA	320/405	1423202/1424052	
4-Methylumbelliferone	355/460	1423552/1424602	
OliGreen	485/538	1424852/1425382	
Phenylalanine-Ninhydrin reaction	390/485	1423902/1424852	
R-Phycoerythrin	544/590	1425442/1425902	
PicoGreen	485/518 or 538	14248852/1425182 or 1425382	
Resorufin	544/590	1425442/1425902	
Rhodamine 110	485/518	1424852/1425182	
RiboGreen	485/538	1424852/1425382	
Rox	578/604	142578206/1426042	
StarBright Green	444/510	1424442/1425102	
Sulforhodamine 101	584/612	1425842/1426122	
Tamra	544/590	1425442/1425902	
Texas Red	584/612	1425842/1426122	
TRITC	544/590	1425442/1425902	
wtGFP	390/510	1423902/1425102	

Ex = Excitation filter; Em = Emission filter

11. Glossary and Abbreviations

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

Chassis The framework of the instrument.

Chemiluminescence A compound that emits light following a chemical reaction is said to be chemiluminescent.

Crossover point The point where the excitation and emission spectra meet.

Crosstalk Interfering signal from neighboring wells.

Crosstalk area The common area for excitation and emission filters used.

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 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.

Filter validation	Procedure in which a new filter pair is introduced to the autocalibration system. The instrument reads the fluorescence of all the reference chips and selects the chip for which the value is most suitable. The selected value is then saved in memory for the autocalibration procedure.
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.
Fluorochrome (Fluorophore)	A molecule or chemical group which emits fluorescence.
Fluorometer	Instrument used for measuring the intensity of fluorescent radiation. Also known as *fluorimeter*.
Fluorometry	Fluorometry is the measurement of fluorescence.
HBW	Half-bandwidth of filters.
Initialization	Initialization tests are so-called self-tests, which are carried out prior to operation to ascertain that the necessary instrument adjustments have been carried out.
LED	Light-emitting diode.
Luminescence	Emission of light (other than from thermal energy causes) such as bioluminescence.
Luminometer	An instrument used for measuring the intensity of luminescent radiation.
Luminophore	A substance which emits light at room temperature. A group of atoms that can make a compound luminescent.
Photomultiplier tube (PMT)	A photoelectric cell that converts light into electric current and amplifies the current.

Reference chip	A filter pair is validated against a reference chip in Ascent Software. If a filter pair does not have a suitable reference chip, the asterisk "*" will appear after the filter wavelengths given.
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.
rpm	Revolutions per minute.
Scaling	The measured values are expressed as RFU or RLU. Scaling is a way to convert readings to show desired values. In fluorometry, the normal beam and the small beam have separate scaling factors. A user-defined scaling factor can be set to the indicated filter pair in Ascent Software (see ***Procedure Desktop/Setup/Filters***). The measured values are then multiplied by this factor.
Self-tests	Initialization tests and adjustments that the instrument performs prior to operation as well as autocalibration.
Stokes shift	The difference between the wavelengths of the excitation and emission peaks.

12. Literature

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13. Warranty Certificate

Thermo Fisher Scientific Microplate Instrumentation Business products are fully guaranteed against defective parts and materials, including defects caused by poor workmanship, for a period of one year from the date of delivery.

Thermo Fisher Scientific will repair or replace defective parts or materials during the term of warranty at no extra charge for materials and labor provided that the products were used and maintained in accordance with Thermo Fisher Scientific's instructions. The warranty is invalid if products have been misused or abused.

For the warranty to be effective, the product must have been purchased either directly from Thermo Fisher Scientific or from an authorized Thermo Fisher Scientific distributor. The guarantee is not transferable to a third party without prior written approval from Thermo Fisher Scientific.

This guarantee is subject to the following exclusions:

- Any defects caused by normal wear and tear.
- Defects caused by fire, lightning, flood, earthquake, explosion, sabotage, war, riot, or any other occurrence of the type listed above.
- Refurbished products that are subject to different warranty conditions.

THIS WARRANTY IS IN LIEU OF ALL OTHER EXPRESSED OR IMPLIED WARRANTIES, INCLUDING BUT NOT LIMITED TO ANY IMPLIED WARRANTIES OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE. The seller is not liable for any loss or damage arising out of or in connection with the use of the product or other indirect damages.

These warranty terms and conditions can be obtained from your local Thermo Fisher Scientific dealer.

This card acts as a warranty certificate.

13.1 Warranty limitations

The following items are not included in the warranty:

- Consumables
- Software programs
- Lamps.

14. Specifications

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

14.1 General specifications

Weight	Basic unit 21 kg (46 lbs.). 3 optional dispensers add 3.5 kg to the weight.
Overall dimensions	420 mm (16.5 in.) (W) x 420 mm (16.5 in.) (D) x 340 mm (13.4 in.) (H), options included.
Operating conditions (indoor use)	+10°C – +40°C, RH 90% max. Tested according to IEC 60068-2-1 test Ab, (Cold). IEC 60068-2-2 test Bb, (Dry heat). IEC 60068-2-3 test Ca, (Damp heat).
Transportation conditions	-40°C – +70°C, packed in transportation packaging. Tested according to IEC 60068-2-1 test Ab, (Cold). IEC 60068-2-2 test Bb, (Dry heat).
Storage conditions	-25°C – +50°C, packed in transportation packaging. Tested according to IEC 60068-2-1 test Ab, (Cold). IEC 60068-2-2 test Bb, (Dry heat).
Mains power supply	100 – 240 Vac, 50/60 Hz, nominal
Power consumption	200 VA max., 32 VA standby.
Heat dissipation	683 BTU max.
Computer interface	Serial RS-232C port. Baud rate 9600. Character format 1 start bit, 8 data bits, 1 stop bit, no parity. Flow control XON/XOFF.
Light source	Quartz-halogen lamp. Power consumption 30 W.

Continued

General specifications Cont.

Detector	Photomultiplier tube.
Fluorescence reference	Lisa KL 3-9402 Polymethylmethacrylate.
Filters for fluorometric measurements	Both the excitation and emission filter wheels can comprise max. eight selectable filters (Fluoroskan Ascent). However, in the Fluoroskan Ascent FL, eight excitation and six emission filters can be installed into the filter wheels, since the emission filter slots 7 and 8 are always reserved for luminometric measurements. Transmittance 30 – 100%, half-bandwidth 6 – 50 nm, depending on the wavelength.
Filters included in the instrument	Excitation filters: 355 nm, and 485 nm. Emission filters: 460 nm, and 538 nm.
Filters for luminometric measurements	Filters can be used. Luminometric filters and fluorometric emission filters are situated in the same filter wheel, maximum 6 filters.
Plate types	1- to 384-well plates. Can also be programmed for nonstandard configurations. Maximum dimensions 90 mm x 134 mm x 25 mm.
Shaker	Built-in orbital shaker with adjustable speed and diameter.
Incubator	Temperature range from RT (25°C) +3°C to 45°C, when the ambient temperature is 25°C. The temperature is selected using Ascent Software.
Dispensers	1 – 3 optional dispensers. Syringe volumes 1000 µl. Dead volume 600 µl. Metal-free fluid path. Exchangeable valve, syringe, tubing and tip. Autoclavable tubing and tip.

14.2 Safety specifications

The Fluoroskan Ascent FL/Fluoroskan Ascent FL fulfils the following requirements:

EN 61010-1:2001
EN 61010-2-101:2002
including CA/US National Differences
CSA C22.2 No. 1010.1 M1992

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 – +40°C
Mains supply fluctuations	± 10% (if larger than specified above)
Installation category (overvoltage category)	II according to IEC 60664-1 (see **Note** 1)
Pollution degree	2 according to IEC 60664-1 (see **Note** 2)

Notes

1) The *installation category* (overvoltage category) defines the level of transient overvoltage which the instrument is designed to withstand safely. It depends on the nature of the electricity supply and its overvoltage protection means. For example, in CAT II which is the category used for instruments in installations supplied from a supply comparable to public mains, such as hospital and research laboratories and most industrial laboratories, the expected transient overvoltage is 2500 V for a 230 V supply and 1500 V for a 120 V supply.

2) The *pollution degree* describes the amount of conductive pollution present in the operating environment. Pollution degree 2 assumes that normally only nonconductive pollution, such as dust, occurs with the exception of occasional conductivity caused by condensation.

Both of these affect the size of the electrical dimensioning within the instrument.

14.3 In conformity with the requirements

Fluoroskan Ascent FL/Fluoroskan Ascent bears the following markings:

Type 374
100 – 240 Vac 50/60 Hz, 200 VA
CE mark
CSA monogram with US designator

Fluoroskan Ascent FL/Fluoroskan Ascent 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

EMC performance:

EN 61000-6-3:2001	Generic emission standard. Residential, commercial and light industry.
EN 61000-6-1:2001	Generic immunity standard. Residential, commercial and light industry.
EN 61326-1:1997 + A1:1998 + A2 (2001) + A3 (2003)	Product family standard.
Test standards	**Performance limits**
EN 55022:1998	Class B, 150 kHz – 1 GHz
EN 61000-3-2:2000	Class A
EN 61000-3-3:1995 + A1 (2001)	
ANSI C63.4:2003	Class B, 450 kHz – 1 GHz; 30 MHz – 1000 MHz
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 C > 95%/5 s, Criteria C 100%/20 ms, Criteria B

14.4 Performance specifications

Warm-up time	< 15 min to rated accuracy.
Measuring speed	Depends on the plate type and the measurement type. The minimum kinetic interval time is 15 s for a 96-well plate (from well A1 back to the same well A1).
Excitation wavelengths	320 – 700 nm
Fluorometric measurement range	Up to approx. 5000 Relative Fluorescence Units (RFU)
Emission wavelengths	Fluoroskan Ascent FL 360 – 670 nm, optional 360 – 800 nm Fluoroskan Ascent 360 – 800 nm
Fluorometric sensitivity	2 fmol Fluorescein in a black 96-well plate, normal beam
Fluorometric dynamic range	> 6 decades Fluorescein in a black Thermo Scientific 96-well strip plate
Luminometric spectral range	270 – 670 nm
Luminometric measurement range	Up to approx. 5000 Relative Light Units (RLU)
Luminometric sensitivity in FL only	40 amol ATP/well using flash reaction, white 384-well plate
Luminometric dynamic range in FL only	> 9 decades over whole gain setting area
Shaker	Orbital method, speed 60 – 1200 rpm, ∅ 1 – 50 mm

Continued

Performance specifications Cont.

Incubator	Heaters: Warm-up time from RT (25°C) to 37°C, 15 min. Liquid in the well: Ambient temperature 23°C, covered 96-well plate, 200 µl/well. Temperature accuracy: Mean temperature of the wells ± 1°C Uniformity: ≤ 1°C Warm-up speed 1 h from 23°C to 37°C on an average 90% of the set value and the ambient temperature.
Dispensers	Dispensing volume 1 – 1000 µl in 1 µl increments. Accuracy ± 3 µl avg. for volumes 5 µl and above Precision 5 – 19 µl < 5% 20 – 1000 µl < 2% Minimum dispensing speed 25 s, 96-well plate, 5 µl/well. The dispenser specifications are given for distilled water at RT.

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Appendix A. Brief User's Guide

- Unpack the instrument (p. 19).
- Check delivery for completeness (p. 20).
- Check for damage during transport (p. 20).
- Place the instrument according to environmental requirements (p. 21).
- Release the transportation lock (p. 22).
- Install the dispenser tubings if equipped with dispensers (p. 25).
- Plug in the instrument (p. 23).
- Switch the instrument ON and ensure that the green light is lit (p. 31).
- Install Ascent Software (p. 31).
- Create an Ascent Software session (see Ascent Software User's Guide: ***Procedure Desktop*/*Session*/*New***).
- We recommend you perform a dummy run first with an empty plate when you have created a new session and then a run with known samples or controls.
- Load the microplate with prepared samples, blanks and controls. Place the microplate onto the plate carrier of the instrument so that the A1 well is located in the upper left corner of the plate carrier (p. 32).
- Start the session by pressing the **START** button on the Procedure Desktop toolbar of Ascent Software.
- Follow the instructions provided by the software.
- Switch the instrument OFF after routine operation (p. 59).
- Maintain the Fluoroskan Ascent FL or Fluoroskan Ascent instrument on a regular basis (p. 61).

Appendix B. Filter Order Form

Thermo SCIENTIFIC

Filter Order Form PO #_____

Filter pairs

Excitation filter [nm]	**Emission filter** [nm]	**Fluorochrome** (fluorescent dye)

Specify the wavelength to the nearest 5 nm.

Provide the excitation and emission spectra of the Fluorochrome, if possible.

Only excitation filter

For single filter order, also specify the emission filter to be used.

Excitation filter [nm]	Emission filter [nm]	**Fluorochrome** (fluorescent dye)

Specify the wavelength to the nearest 5 nm.

Provide the excitation and emission spectra of the Fluorochrome, if possible.

Filter Order Form Cont.

Only emission filter

For single filter order, also specify the emission filter to be used.

Excitation filter [nm]	**Emission filter** [nm]	**Fluorochrome** (fluorescent dye)

Specify the wavelength to the nearest 5 nm.

Provide the excitation and emission spectra of the Fluorochrome, if possible.

List of standard fluorometric filters:

Excitation: 320 nm, 355 nm. 390 nm, 430 nm, 440 nm, 444 nm, 485 nm, 530 nm, 544 nm, 578 nm, 584 nm, 646 nm

Emission: 405 nm, 440 nm, 460 nm, 485 nm, 510 nm, 518 nm, 520 nm, 527 nm, 538 nm, 555 nm, 590 nm, 604 nm, 612 nm, 620 nm, 678 nm, 680 nm

Thermo Fisher Scientific contact information:

e-mail to your sales representative or fax to +358-9-3291 0580

Appendix C. Addresses

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

http://www.thermo.com

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Distributed by:

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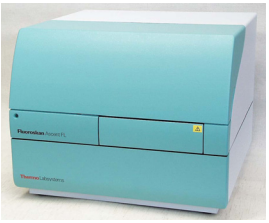

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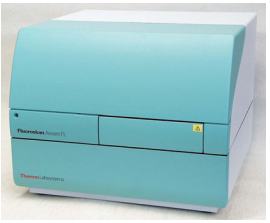

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