# S 5200
# Autosampler
# User Manual

**Version 3.2**

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# 1. STANDARD ACCESSORIES

| | |
|---|---|
| 1 piece | Drain Tubing (Silica) |
| 1 piece | Bottle for washing solutions; volume 500 ml; with Teflon tubing and fitting connector |
| 1 piece | Bottle for waste; volume 1000 ml; with Teflon Tubing and fitting connector |
| 2 pieces | Standard Sample Racks (for 60 x 1.5 ml vials) |
| 1 piece | Power Cable |
| 100 pieces | Sample Vials (1.5 ml or 2 ml) |
| 100 pieces | Screw Caps with Septum |
| 2 pieces | Fittings with ferrules (stainless steel or PEEK) |
| 2 pieces | Capillaries I.D. 0.25 mm (stainless steel or PEEK) |
| 1 piece | Remote Control cable w. Connector |
| 1 set | Fuses |

The accessories enclosed can vary, depending on the version of Autosampler ordered.

## 1.1 VERSIONS & UPGRADE OPTIONS

The Autosampler S 5200 is available in 3 different versions and can be upgraded with several options.

- **Fixed Volume Injection Version (Standard Version)**

  The standard version is delivered with 20 µl sample loop and 500 µl syringe. The parts coming into contact with the liquids are made of stainless steel. For special application, the sample loop and syringe can be exchanged.

  With programmable eluent segment to ensure that the sample segment is transported completely into the sample loop.

  With programmable air segment to keep the eluent and sample segment apart. For the injection of larger volumes (e.g. for semi or preparative applications) the two sample tacks can be exchanged for rack suitable for 5 ml vials. For smaller volumes, micro titer racks are also available.

- **Variable Volume Injection Version**

  The Variable Volume Injection version is delivered with a 100 µl sample loop which can be partially filled.

§ **Derivatisation Version**

Before injection, each individual sample can be mixed with up to 3 different reagents. A reaction time as well as a washing cycle between the dosage of the single reagents is programmable.

§ **Chemically Inert Option**

For aggressive samples or especially with sensitive substances, the biocompatible S 5200 PEEK version is recommended. All components which come into contact with the sample and/or eluent (e.g. sample needle, valve, and capillaries) are made of Polyetheretherketone (PEEK).

§ **Heating/Cooling Option**

For storage of unstable samples the Autosampler S 5200 can be upgraded with a cooling/heating device. The sample racks can be cooled/heated very accurately between 5° and 70° C with built-in Peltier elements.

# 2. SAFETY INSTRUCTIONS

The manufacturer does not warrant for any defects or damage resulting from incorrect operation and maintenance, non-observance of the manual's instructions and negligence during installation.

Before putting the instrument into operation, read the manual carefully and should there be any further questions, please get in contact with your supplier.

## 2.1 GENERAL ELECTRICAL HAZARDS

- Check actual line voltage to confirm that the set voltage (on the rear side; voltage selector and fuse carrier) of the instrument is correct.
- Before changing the instrument's voltage or before changing defect fuses, disconnect the instrument from all power sources.
- The instrument has to be plugged into grounded wall sockets only.
- This instrument can be used only with other instruments which comply with the general safety regulations.
- Connect all cable connections before switching on the instrument.

## 2.2 GENERAL PRECAUTIONS

- In order to avoid any damages, perform periodic leak checks on all installed supply lines.
- The instrument is only allowed to be used for applications with specifications described in this manual.
- For flammable and/or toxic solvents, follow a regulated and approved waste disposal program. Never dispose of such products through the municipal sewage system.
- The instrument is suitable for operation between 10° C and 35° C surrounding temperature.
- Through improper handling the needle can be damaged or broken. Also please be aware of the danger of injury when putting your hand into the sample chamber during operation!

| | |
|---|---|
| **ATTENTION:** | **Only work in the sample chamber when the instrument is not in operation!** |

- For trouble-free and reliable operation, it is recommended to use only filtrated (0.45 µm) or particle-free samples for the degassing process.

- The standard model S 5200 comes with a needle of stainless steel. To avoid corrosion, the ph value of the sample should be between 2 and 11 (Chemically inert version of the needle is optionally available).
- The wash solution and eluent used for the analysis have to be degassed before operation.
- The instrument should be cleaned only with appropriate cleaning agents.

# 3. TECHNICAL SPECIFICATIONS

| | |
|---|---|
| **Sampling** | X-Y-Z-Operation; stepper motor driven syringe |
| **Control** | integrated microprocessor (Motorola 68000) |
| **Dialogue** | selectable between English and German |
| **Communication** | RS-232 interface and interface for system control (remote control); 5 integrated REED relays: inject marker and/or remote control of external components |
| **Sample Loop** | exchangeable; standard volume: 20 µl (other volumes available) |
| **Sample Dosage** | dosage through stepper motor driven 500 µl glass syringe |
| **Material** | stainless steel (optional: PEEK) |
| **Reproducibility** | < 0.5 % with 100 µl fixed volume injection method |
| | < 1.0 % with 20 µl variable volume injection mode |
| **Memory Effect** | < 0.01 % (dependent on selected washing program) |
| **Washing Program** | programmable wash cycles and volume |
| **Analysis Time** | up to 9999 programmable; time base selectable in 1 min., 0.1 min. or in seconds |
| **Sample Racks** | standard: 2 racks for 60 vials (1.5 ml) each; other racks on request |
| **Power Requirement** | 230 V, 110 V; 50/60 Hz |
| **Fuses** | 230 V: 2x 1.0 AT |
| | 110 V: 2x 2.0 AT |
| **Working Temperature** | 10 – 35° C |
| **Dimensions** | 415 x 300 x 445 mm (H x W x D) |
| **Weight** | 20 kg |

# 4. INSTALLATION

## 4.1 LOCATION

All components are single elements which can be individually used or combined to a complex module system.

This ensures an optimum system arrangement of the individual instruments, depending on the application requested. For the Autosampler S 5200 a space of 350 x 450 mm is required. It is recommended that the rear side as well as the right side of the instrument remains accessible for exchanging the wash solvent and/or sample loop.

## 4.2 TRANSPORTATION SAFETY

For transportation purposes, a safety catch has been mounted on the moving sample arm. Before starting the instrument, remove this safety catch:

- Open the Autosampler's cover
- remove the red safety catch by removing the 4 screws
- remove the red safety band by cutting it
- close the cover

Before any transportation of the instrument, mount the safety catch again to avoid damage to the instrument!

## 4.3 INSTALLATION

*NOTE: When using the Autosampler S 5200 with the Amino Acid Analyzer S 433, please refer to the **Amino Acid Analyzer Installation Manual**.*

### ELUENT CAPILLARY

Connect the eluent capillary (stainless steel or PEEK) with the appropriate fittings and ferrules to the connection "PUMP" positioned at the front panel of the instrument. The other end of the capillary has to be connected to the pressure side of the pump head.

### SAMPLE CAPILLARY

Connect the sample capillary to the connection "COLUMN" on the front panel of the instrument.

### RACK FOR WASH SOLUTION

Install the rack enclosed with the pre-mounted screws at the rear panel of the instrument.

## BOTTLE: WASHING SOLUTION

Fill the 500 ml bottle with ***completely degassed*** washing solution. Place it on the appropriate rack on the backside of the instrument and close the bottle. Connect the Teflon tubing to the backside connector "WASH SOLUTION" below the bottle rack.

## BOTTLE: WASTE

The 1000 ml bottle is used for collecting the waste. It has to be positioned on the same level as the autosampler to ensure trouble-free transportation into the waste bottle. The tubing has to be connected with the ferrule and fitting to the connection DRAIN on the back side.

## SILICA TUBING

At the rear side of the instrument, a connecting piece for removing the wash solvent (when using the PURGE PORT for flushing the needle) is installed. Slip the Silica tubing onto this connection and position the other and of the tubing into a lower placed water reservoir.

## SAMPLE RACKS

Place the sample rack 1 (positions 1 – 60) at the sample chamber's left side and rack 2 (61 – 120) at the right side. The pivots mounted to the racks bottom side have to snap into the appropriate fixtures.

## REMOTE CONTROL

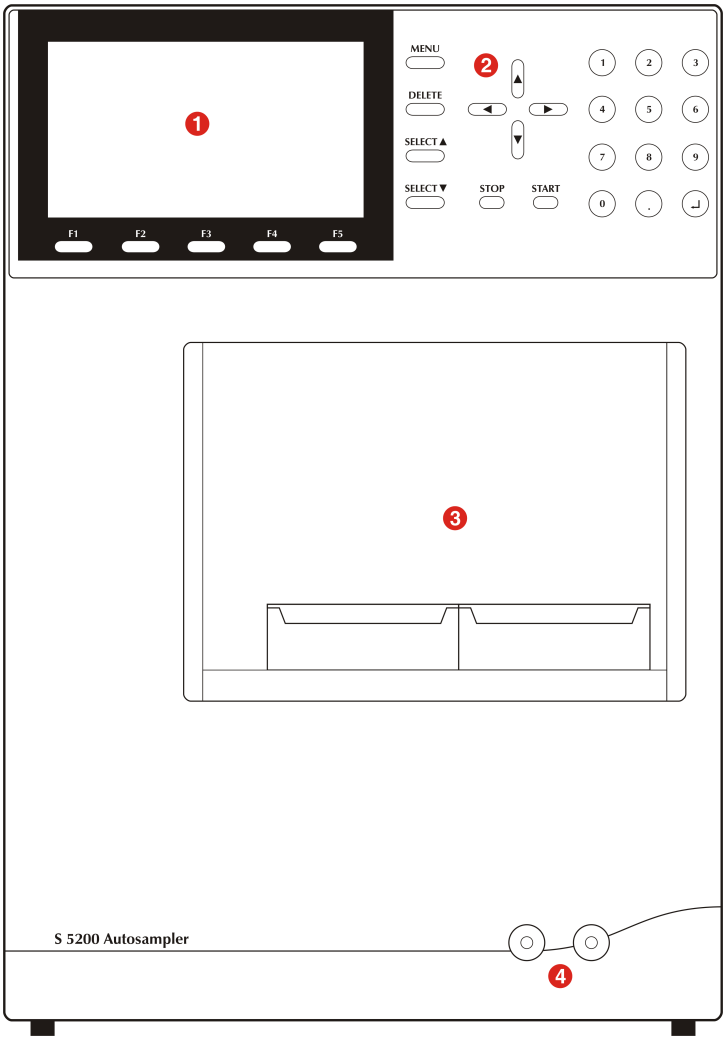
Please refer to the functions of the 9pin Sub-D connector to ***10.6.1 Remote Control***.

# 5. GENERAL DESCRIPTION

## 5.1 FRONT SIDE VIEW

All parts necessary for the operation are positioned on the front side of the instrument.

Via the large LCD all functions can be controlled at any time. The integrated keyboard makes it possible to program the pump via the menu for nearly all analytical applications.

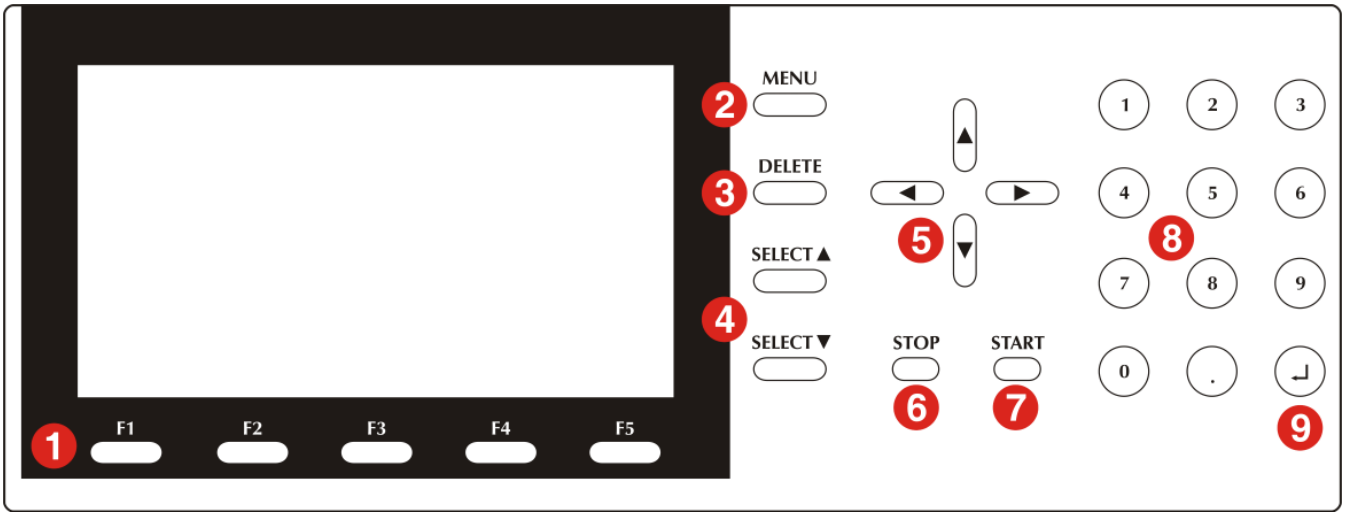



**Picture 1: Front Side View**

1 Large Graphical Display
2 Keyboard
3 Sample Chamber w. Sample Racks
4 Capillary connections (not on Amino Acid Analyzer variant)

## 5.1.1 OPERATION PANEL

The programming and control of the pump is done by a stepwise dialog, using different menus. With the Function Keys [F1] – [F5], positioned below the display, different menus/functions can be selected directly depending on the current menu. With the numeric keys, values are entered.



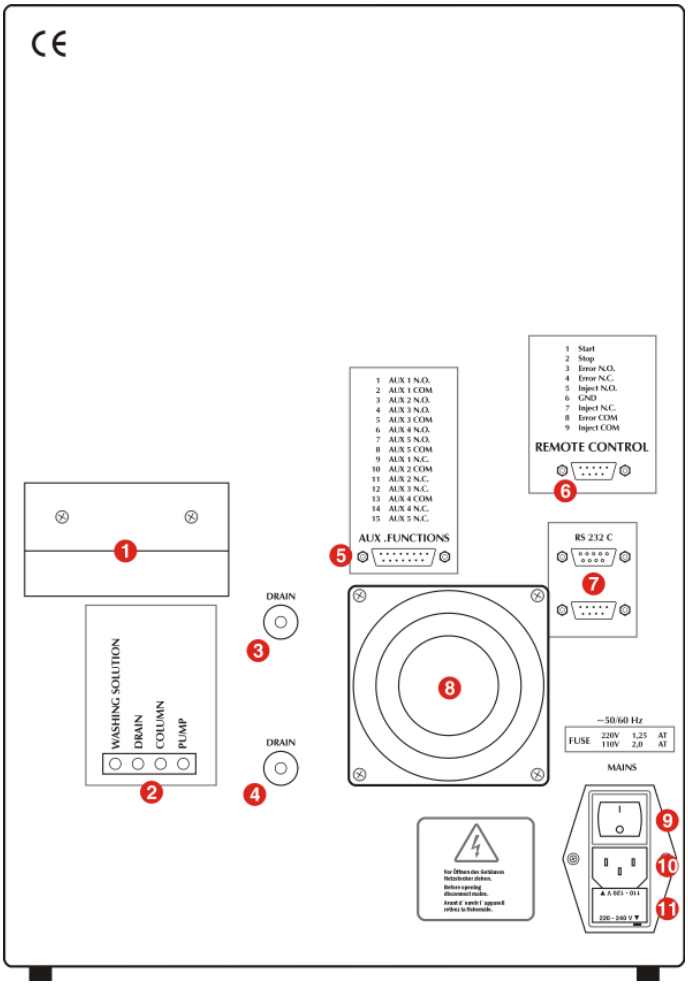

**Picture 2: Operation Panel**

| | | |
|---|---|---|
| 1 | Function keys [F1]...[F5] | The actual function of the keys is shown on the display. These are dependent on the current menu selected. |
| 2 | [MENU] key | With this key, it is possible to select the main menu at any time |
| 3 | [DELETE] Key | Deletes the current value |
| 4 | [SELECT] keys | For selecting variable settings in the individual menus |
| 5 | [CURSOR] keys | For navigating through the display menus |
| 6 | [STOP] key | Stop/Hold the instrument: |
| 7 | [START] key | Start the instrument |
| 8 | Numeric Keys [0]..[9] | Used for entering numeric values |
| 9 | [ENTER] key | Used for entering values |

# 5.2 BACK SIDE VIEW

At the rear side of the Autosampler, the communication part is installed, like the serial communication port RS-232C, Interface for the operation with a PC and an additional connection for the remote control of external components. Due to these connection possibilities, the autosampler can be integrated in totally different analysis systems.

At the back, the bottle for the wash solvent, necessary for flushing the system as well as the drain for not needed sample solution or used wash solution is positioned.




**Picture 3: Back Side View**

1 Wash Bottle Rack
2 Capillary Connections
3 Connection DRAIN (only w. Cooling option)
4 Connection DRAIN
5 Aux. Functions connector
6 Remote Control Connector
7 RS-232 Serial Connector
8 Cooling Fan (only w. Cooling option)
9 Power Switch
10 Power Cable Connector
11 Fuse Carrier w. Voltage Selector

# 5.3 CAPILLARY CONNECTIONS

## WASH SOLUTION

The 500 ml bottle for the wash solvent is placed into the special rack at the rear side of the instrument and has to be filled with the degassed wash solution. The Teflon capillary has to be mounted with the fitting to the WASH SOLUTION connection. The capillary must be put through the bottle's cap hole into the solution.

Note: To ensure a correctly done wash cycle, the bottle has to be filled with the wash solution and the end of the capillary has to reach into the wash solvent.

For most applications, the eluent can be used as wash solvent.

Important: The wash solvent/eluent has to be completely degassed before operation.

Even smallest gas bubbles, especially with very low sample volumes, can cause incorrect dosage thus causing poor reproducibility of the analysis results.

## SILICA CAPILLARY

The silica capillary with the inside diameter of approx. 5 mm must be slipped onto the connection DRAIN (3 and 4). The end of the capillary should end in a lower placed drain reservoir.

## DRAIN

This drain is used for removing not needed samples and washing solution during flushed loop injection method.

The Teflon capillary has to be fitted with the fitting and the ferrule to the connection 7 and has to lead into the waste bottle (1000 ml) positioned at the same height as the instrument.

The waste bottle has to be emptied before the fluid level is even with the flow capillary.

# 5.4 CABLE CONNECTIONS

## INTEGRATION INTO AN ANALYSIS SYSTEM

The Autosampler is integrated into the system via the RS-232 interface or D-Sub connector (REMOTE CONTROL) and can be controlled via contact closures. Information like error messages or injections performed can be detected at the output connector.

Following functions are possible:

- Start of sample sequence
- End of sample sequence
- Error message
- Message about the injection performed

# REMOTE CONTROL OF EXTERNAL COMPONENTS

In case, the autosampler is integrated into a system without any PC, the autosampler can be used for the control of the other components.

Five potential-free relay contacts can be programmed in a separate sub-menu (AUX.FUNCTION Menu). As control signal, the relay uses a time controlled signal or a programmed impulse. Load of the relay when controlling external instruments:

Max. Voltage: 175 V

Max. Power: 3.0 W

Max. Current: 0.25 A

# CONTROL VIA RS-232 INTERFACE

With the help of the RS-232 interface (3), the autosampler can communicate with a PC.

The RS-232 connector has to be cabled with the RS-232 connector of the PC. For starting the data acquisition, the inject signal (Remote connector) has to be connected as a digital signal to the start input of the PC.

# 6. INSTRUMENT DESCRIPTION

## 6.1 GENERAL

The Autosampler S 5200 is an automatic sample processing system; the standard model can inject up to 120 samples. The instrument can be used as a stand alone system and can be also integrated into a system controlled via PC. The analysis program can be easily adjusted to the individual application through the different menus.

With the fixed volume injection version, the sample volume is dependent on the built-in sample loop, which is easily exchangeable.

With the variable injection version, the stepper motor driven syringe measures exactly the sample volume selected. The sample loop installed will be filled only partially with the selected sample volume and after turning the valve, is transported to the separation column. This injection technique allows the accurate dosage of the sample volume.

A programmed sample volume is sucked via the sample needle into the system. Between the sample needle and the syringe, a transfer capillary is installed, which holds the measured sample volume until injection. This avoids that any sample comes into the syringe which could cause any contamination. For transportation of the measured sample volume into the injection system, the sample needle is lowered into the inject port and is pushing the sample volume into the sample loop. To secure the complete transfer of the sample segment, a programmable eluent segment can be sucked on by the syringe. With this eluent segment, placed in front of the sample segment, the sample can be transferred into the sample loop without any loss. After the injection, the eluent segment fills completely the capillary between the injection port and sample loop. To avoid any mixing of the sample with the eluent segment, an air segment can be programmed which keeps the two segments apart.

For standard application, a sample loop of 20 µl is connected to the switching valve.

The Autosampler with variable injection mode can be used for both - fix volume and variable volume - injection operation. When using the fix volume injection method, an appropriate sample loop has to be installed.

On request, the instrument can be upgraded for other applications:

- Pre-column derivatisation: up to three independent reagents can be added to each sample, mixed with the sample and programmed with independent reaction times. In addition, the sample can be diluted (up to 1/100) before injection.
- For temperature sensitive samples, the sample tray can be cooled or heated with an optional cooling system. The heating/cooling process is done via built-in Peltier elements.
- For chemical inert operation, the instruments' parts like needle, capillary, valve unit etc. are made of PEEK and FEP materials.

- A wide range of sample racks for different sample vials can be used. Racks from 1 µl up to 5000 µl or micro titer plates are available.

# 6.2 SYSTEM DESCRIPTION

## 6.2.1 MECHANICS

All parts of the instrument (cover, sample racks, sample needle etc.) which may come in contact with aggressive substances are made of stainless steel and other resistant materials.

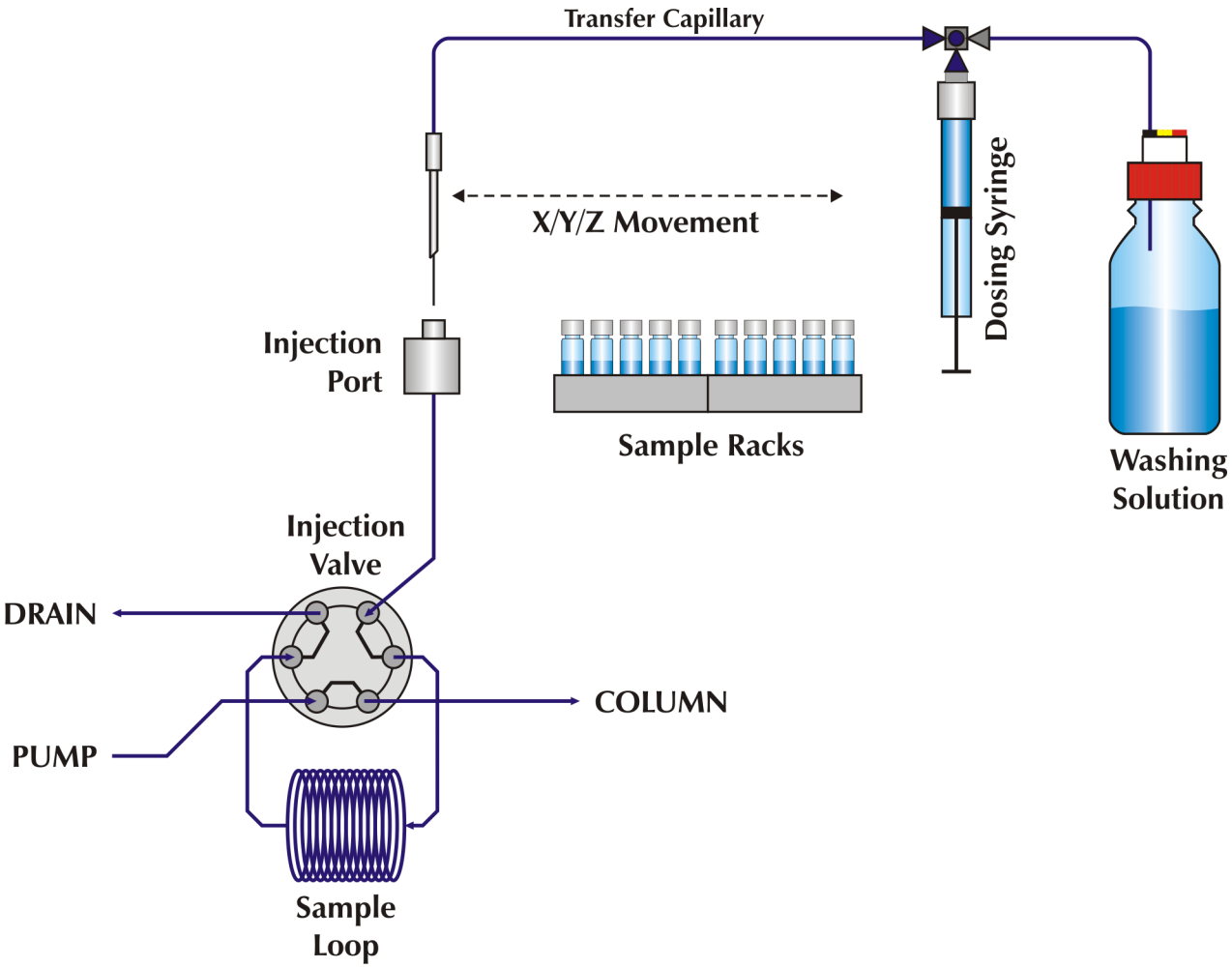
For certain applications (e.g. Ion Chromatography) where extremely strong acids or concentrated saline solutions are used as eluent, the sampling part is made in PEEK.

The sampling process is done with a stepper motor driven sample needle (X, Y, Z axis). For the exact needle inject position, each shaft is controlled by a separate stepping motor.

All the shafts are moved via linear slideways.

## 6.2.2 SAMPLING PART

The injection system of the Autosampler consists of the following six important units:




**Picture 4: Sampling Part**

- **The Sample Needle** mounted to guide bars is stepper motor driven in X, Y, Z axis. The needle is moved to the individual sample vial, lowered, the septum pierced through and the sample taken.
- **The Transfer Capillary** between the sample needle and syringe. A pre-selected sample volume is measured exactly by the syringe and sucked into the capillary.
- **The 3/2-Way-Valve** is positioned above the syringe. For sucking on sample volume/air segment or wash solvent/eluent segment, the valve switches automatically.
- **The Syringe** where the piston is moved up and down with a linear driven stepper motor. The selection of the stepping motor's steps ensures the accurate measurement of the volume to be sucked on.

- **The Wash Solvent**; the syringe sucks on a programmed volume of wash solvent for flushing the syringe and the transfer capillary. The wash solvent is pushed into the inject port via the sample needle.
- **The sample valve with the sample loop**, where the sample is injected into the system and transported together with the eluent to the separation column.

### 2.6.2.1 SAMPLE NEEDLE

The needle is mounted to a sliding carriage and is moved through step motors in X-Y direction. When the needle is directly above the selected sample vial, it will be lowered down (Z-direction). This is done by lowering the sliding carriage where the needle is mounted to. The needle pierces through the septum. The sample is sucked into the transfer capillary between needle and stepper motor driven syringe. However, if no vial was placed into the selected vial position, the vial sensor plate activates an optical sensor. The exact vial position error will be displayed.

### 2.6.2.2 TRANSFER CAPILLARY

The transfer capillary has a certain length and diameter. This ensures that the sample remains in the capillary only and will not be sucked into the syringe. For flushing the system, only the capillary and the needle has to be flushed, not the syringe.

### 2.6.2.3 3/2-WAY SWITCHING VALVE

The switching valve is mounted on top of the syringe. Depending on the switching position, sample/air segment is sucked into the transfer capillary or wash solvent/eluent segment is sucked into the syringe.

### 2.6.2.4 SYRINGE

Sample as well as wash solvent is exactly measured and transported by the syringe. For certain applications, the standard syringe (500 µl) can be exchanged by a syringe of different volume (max. 5000 µl)**.** For sampling, the needle is moved to the vial, lowered down and the programmed sample volume sucked in by the syringe**.** Accurate dosage is possible due to the large amount of steps (17 steps/µl with a 500 µl syringe). For the following wash cycle, the 3/2 way valve switches to the wash solvent and the syringe sucks on the wash solvent. After the wash cycle, the valve is switching back to the transfer capillary, connected to the needle. The wash solvent present in the syringe is pushed out and transported through the transfer capillary and needle to the purge port or inject port.

**Important:** For reliable operation, no air bubbles should come into the syringe. Should there be any air bubbles present in the syringe; they have to be removed through several wash cycles or by flushing with organic solvent.

## 2.6.2.5 WASH SOLVENT BOTTLE

For flushing the system before the next injection is carried out, a wash cycle program can be selected. The 3/2 way valve switches to the wash solvent capillary and the syringe sucks on the volume programmed from the wash solution bottle. With most applications, the eluent is also used as washing solution.

## 2.6.2.6 SAMPLE VALVE

For transporting the sample into the eluent stream, a motor driven 6 position injection valve is used. With this valve, the sample is injected in the sample loop and together with the eluent stream transported to the separation column. With the fixed volume injection method, the installed sample loop is overfilled with the sample (refer to above drawing: pos. 1 and pos. 4).

With variable volume injection mode, an oversized sample loop is partially filled with the sample volume selected.

Turning of the valve's rotor will switch the sample loop into the eluent stream and the sample volume is transported together with the eluent to the column.

When the sample loop is to be filled, the rotor of the 6-way switching valve is switching to:

- Port 1 and 6: Connecting inject port and sample loop
- Port 4 and 5: Connecting sample loop with the drain
- Port 2 and 3: Direct connection of HPLC pump to the column

For the injection of the sample, the valve is switched. Now the eluent is directly transported through the sample loop. The sample dosed via the sample loop is transported together with the eluent stream to the column. Hereby, the rotor switches to:

- Port 3 and 4: The HPLC pump is connected to the sample loop
- Port 1 and 2: The end (exit side) of the sample loop is directly connected to the column
- Port 5 and 6: connected

The sampling procedure is followed up by the flushing cycle. Hereby, the sample needle and the injection valve are flushed with wash solvent to avoid any transfer of sample remaining.

## 2.6.2.7 FIX VOLUME INJECTION/VARIABLE INJECTION MODE

The Autosampler S 5200 with variable injection mode can be used also for fix volume injection mode.

**Fix volume injection:**

The sample volume is dependent on the size of the sample loop which will be overfilled with sample. Turning of the valve's rotor, will switch the sample loop into the eluent stream and the sample volume is transported together with the eluent to the column. Hereby, unique accuracy and reproducibility is achieved. The disadvantage of this method, the sample loop has to be changed when injecting different sample volumes. Also, there will be a loss of sample substance caused by the overfilling of the sample loop.

**Variable volume injection:**

The measurement of the sample volume is done by the syringe. The sample segment is transferred into an oversized sample loop (partially filled). Due to the high resolution of the syringe's stepper motor (17 steps/µl with a 500 µl syringe), precise dosage and good reproducibility of the analysis results is possible with this operation method. The loss of sample substance can be neglected due to the accurate syringe dosage and the programmable eluent/air segment.

# VARIABLE INJECTION MODE

The standard version of the Autosampler S 5200 comes with a sample loop of 100 µl. To ensure accurate dosage, the syringe sucks on a certain volume of eluent. To keep the sample and eluent apart, an air segment can be programmed which will be sucked on through the needle. Afterwards, the sample is sucked on by the stepper motor driven syringe.

**Even small gas bubbles can affect the exact measurement of the sample volume. Eluent and wash solution have to be degassed before operation!**

# FIX VOLUME INJECTION MODE

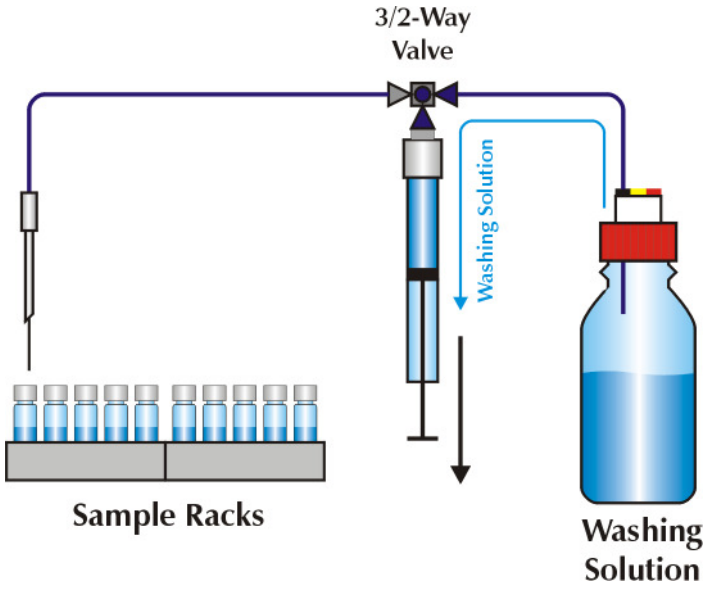
The sample volume is dependent on the sample loop installed. With this method, a distinct surplus of sample has to be programmed (1.2 - 5 times of sample loop volume). To reduce the sample loss, an eluent segment can be programmed which will be sucked on before the sample segment. In order to keep the sample and eluent apart, an air segment can be programmed.

**Even small gas bubbles can affect the exact measurement of the sample volume. Eluent and wash solution have to be degassed before operation.**

# 6.3 WORKING PRINCIPLE (VARIABLE)

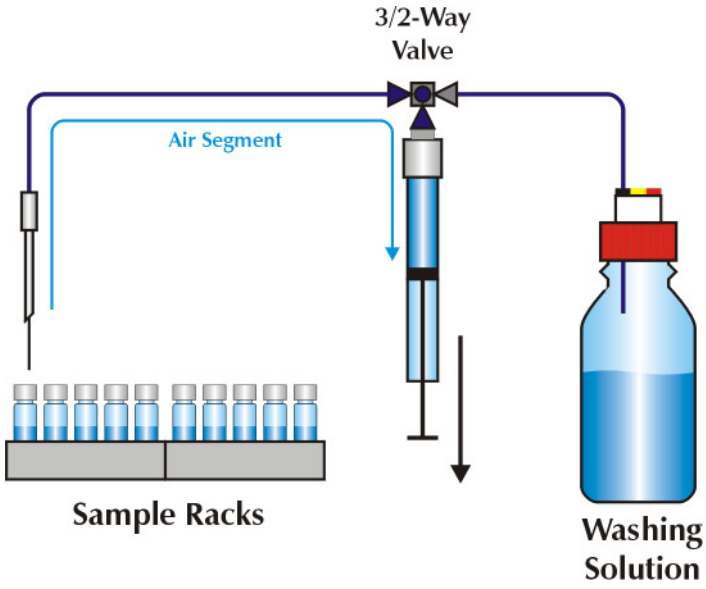
## 6.3.1 TAKING THE SAMPLE

The sampling unit is moved to the selected sample vial with the help of stepping motors. First, the programmed eluent volume is sucked from the wash solvent bottle into the syringe.



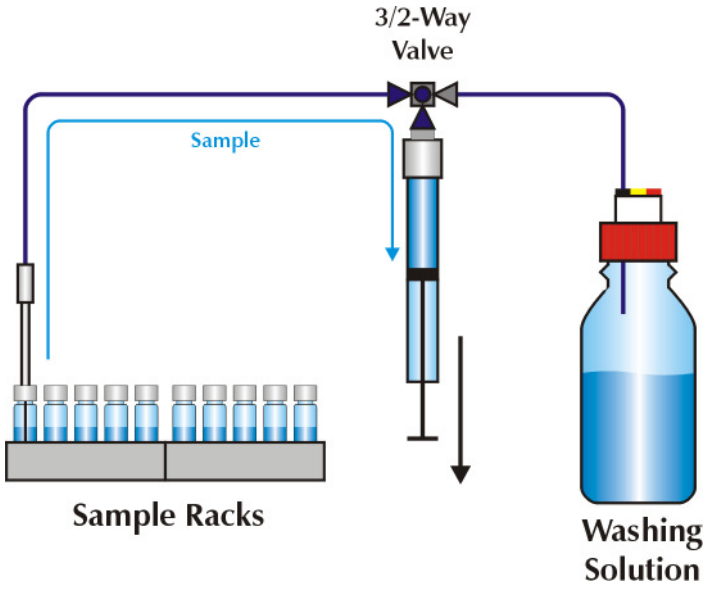

**Picture 5: Working Principle - Eluent Segment**

Then, the 3/2-way valve, positioned above the syringe, switches to connect the syringe to the sample needle. Now, the desired air volume - used for keeping the eluent and sample from each other apart - is sucked on.




**Picture 6: Working Principle - Air Segment**

Now, the needle is lowered down to the level programmed and the required sample volume (+Additional Volume programmed in Service Menu #3) is sucked on. After the needle moves up again, a second Air Segment is taken.




**Picture 7: Working Principle - Sample**

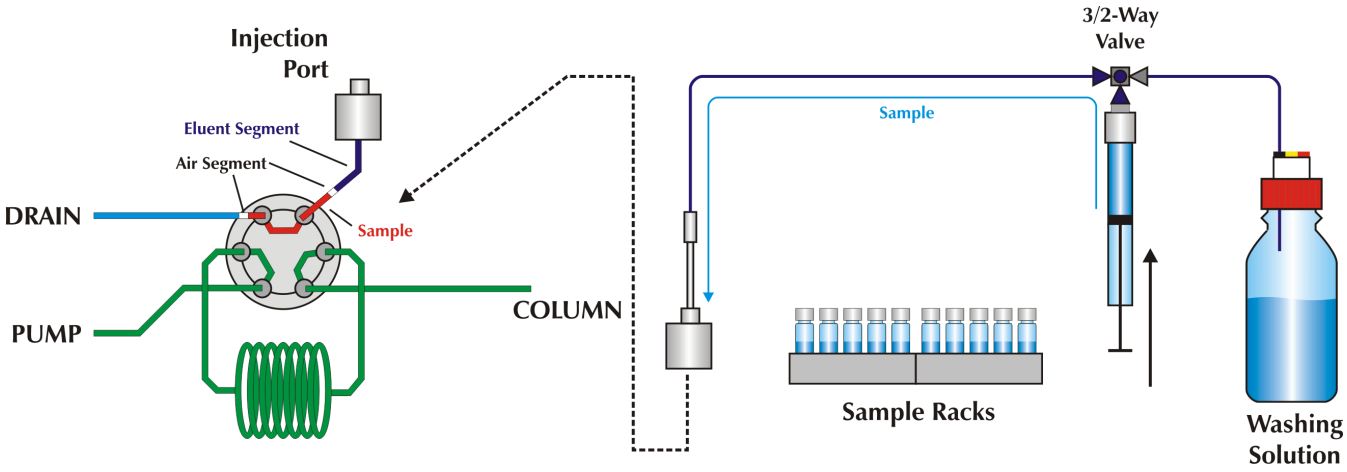
**Note: The max. volume of the transfer capillary is 250 µl. In case of higher volumes, the autosampler has to be modified.**

The length of the transfer capillary is exactly calculated, so that the sample remains only in the capillary and is not sucked into the syringe. Contamination or time consuming wash procedures of the syringe are eliminated.

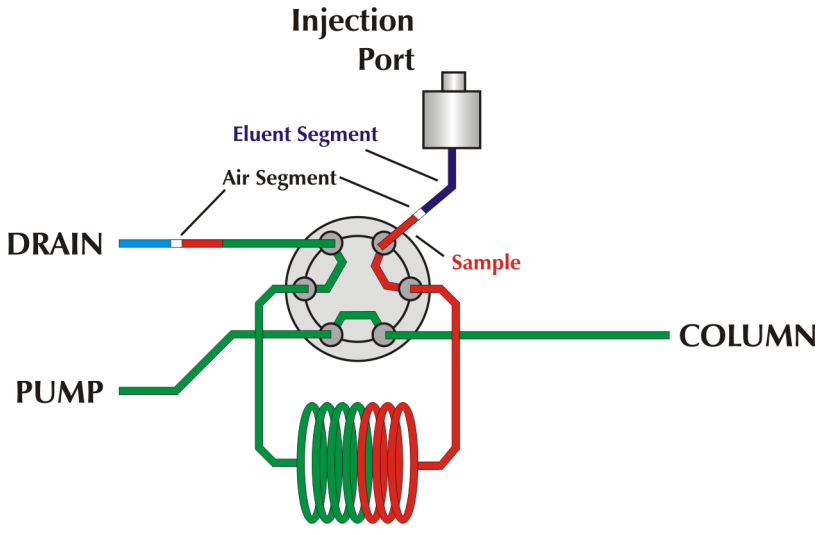
## 6.3.2 TRANSFER TO THE SAMPLE LOOP

The sample needle is positioned above the inject port and lowered into this port. Hereby, the needle is hermetically sealed through a PTFE seal. Via the syringe, the sample is pushed into the injection port till the injection valve. The eluent and air segment sucked in before remains in the capillary between the injection port and the injection valve. The volume of this capillary is approx. 28 µl. Now, the injection valve is switched to the load position and the programmed sample volume (e.g. 20 µl) is injected into the sample loop. This way no wash solution or air can enter the sample loop.

The air and eluent segment can be programmed in the analysis menu.



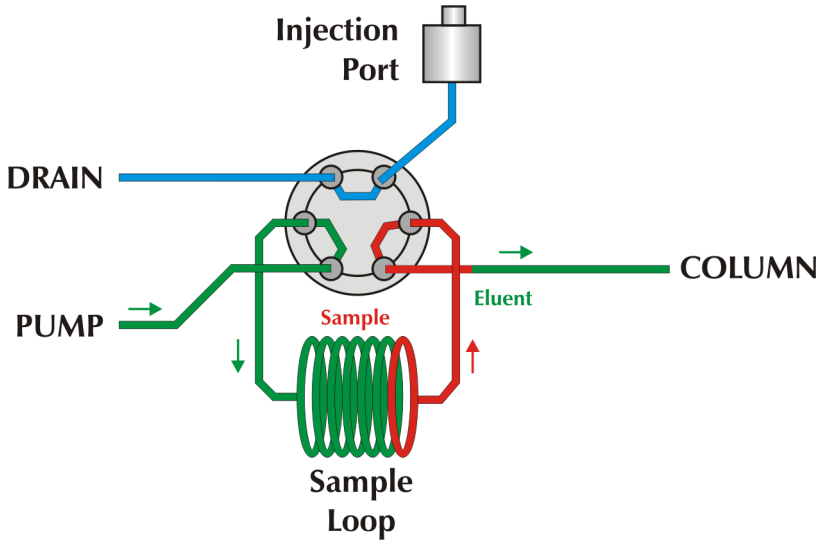

**Picture 8: Working Principle – Transfer to the Sample Loop**




**Picture 9: Working Principle - Filling the Sample Loop**

## 6.3.3 SAMPLE INJECTION

After the sample loop filling process is finished, the rotor of the injection valve is switched (60° turn) and the sample loop with the HPLC pump/separation column connected. The eluent is now directly transported through the sample loop and flushes the sample loop volume to the separation column. The injection valve remains in this position until the analysis is finished. That means, that the sample loop is constantly flushed with the eluent.




**Picture 10: Working Principle - Sample Injection**

Switching of the injection valve for transferring the sample to the column

With fix volume injection, the injection and dosing steps are analogue and the sample overflow will be transported into the waste reservoir.

## 6.3.4 WASHING CYCLE

To avoid any transfer of sample material, all parts which come in contact with the sample should be flushed after each injection.

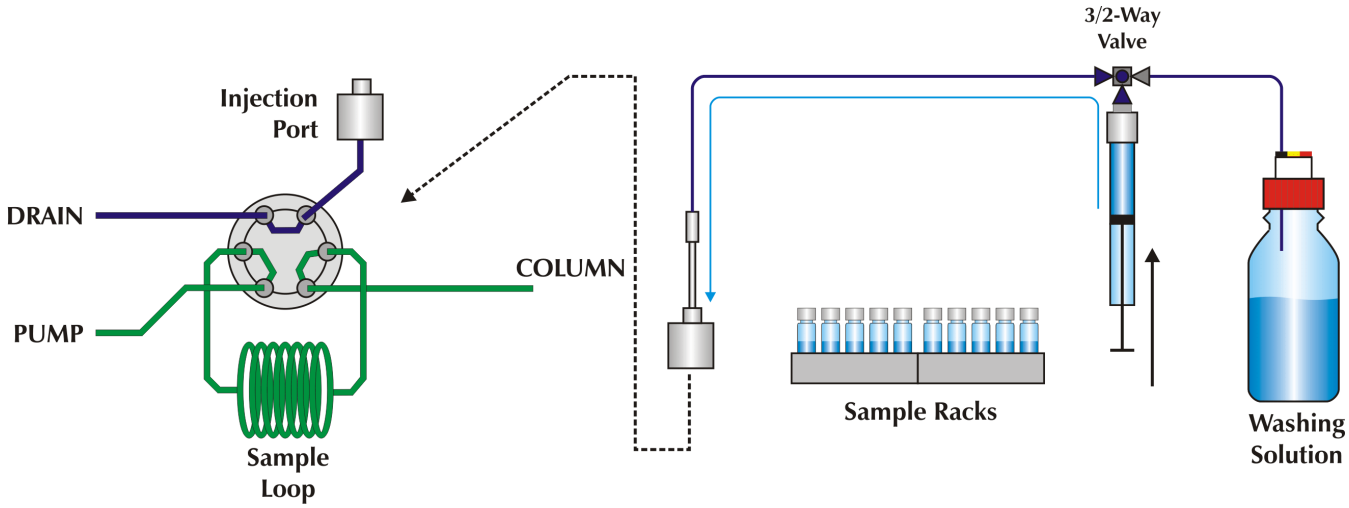
This can be done through the separate needle wash port or via the injection unit.

If the washing cycle is carried out via the purge port, the inside as well as the outside of the needle will be flushed. Generally, this is not necessary, as the sample present at the outside of the needle is sufficiently enough removed through the septum of the vial. Only certain special applications need the inside and outside flushing procedure.

If the washing cycle is done through the injection unit, all capillaries which came in contact with the sample are flushed and washed.

Therefore, in most cases, the injection unit is selected for carrying out the washing procedure.

For the washing cycle procedure, the syringe used for the sampling is applied. The total volume of the needle is filled with wash solvent via the 3-way switching valve. The wash solvent is then transferred through the needle to the drain port or through the injection unit to the drain.



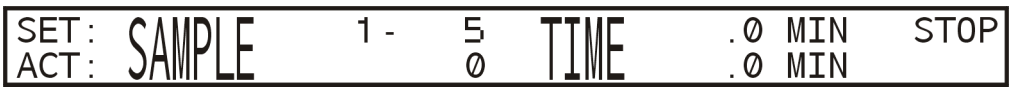

**Picture 11: Working Principle - Wash Cycle**

# 7. SCREEN MENUS

All menu screens have a uniform design and are divided into 3 parts: the status bar, the function key area, and the content area.

## STATUS BAR

The status bar is always displayed on the upper part of the screen, regardless of which menu is currently active.




**Picture 12: Status Bar**

The status bar displays the following information:

- Sample Range (SET)
- Current Sample (ACT)
- Analysis Time
- Run Status
- Error Code

## FUNCTION KEY AREA

The function key area is displayed on the bottom part of the screen. This area shows the function of the corresponding key [**F1**] to [**F5**]. The actual function of each key depends on the current menu. An empty function key area of a specific key denotes no assigned function in the current menu.


**Picture 13: Function Key Area**

## CONTENT AREA

The content area is the area between the status bar and the function key area. It shows the actual menu content with text and parameters.

This is the region where parameter changes can be done. Navigating through the content area is done with the [**CURSOR**] keys and the screen cursor is updated accordingly.

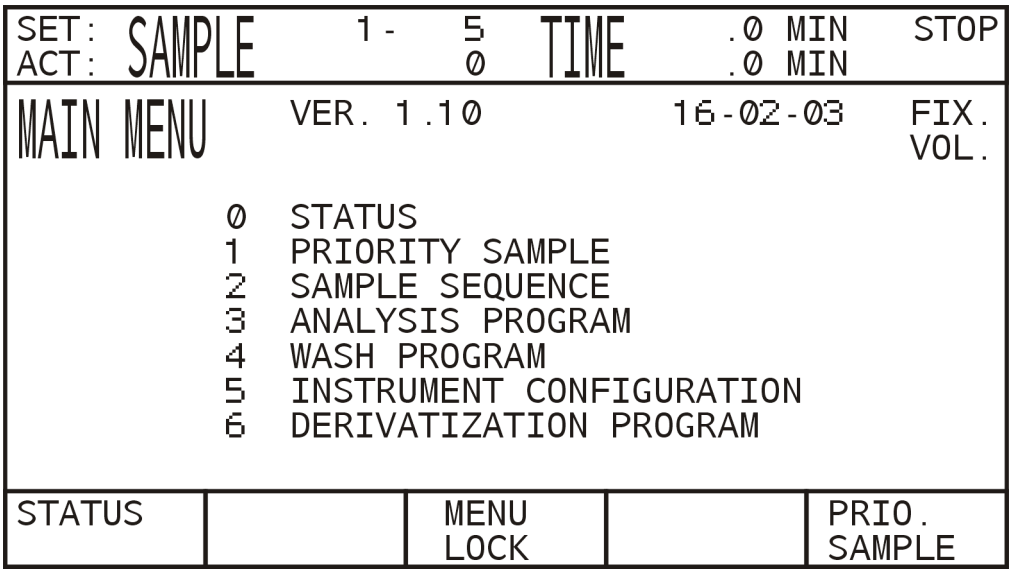
## CHANGING PARAMETERS

Changing parameters within a specific menu is done as outlined below.

- move the screen cursor to the value you want to change by using the [**CURSOR**] keys.
- enter the new value by using the numeric keys [**0**]..[**9**], or the [**SEL_UP**] and [**SEL_DOWN**] keys, depending on the kind of value. Whenever a value is selected and a "?" is displayed, the value can be edited only with the [**SEL_UP**] and **[SEL_DOWN**] keys.

# 7.1 MAIN MENU

This menu can be displayed at any time by pressing the [**MENU**] key at the front panel.




**Picture 14: Main Menu**

## DESCRIPTION

You can select any of the displayed menus by moving the screen cursor to the appropriate menu using the [**CURSOR**] keys and pressing the [**ENTER**] key. Alternately, you can use the numeric keys [**0**] to [**6**] to access the menus directly.

The following menus can be access from the MAIN MENU:

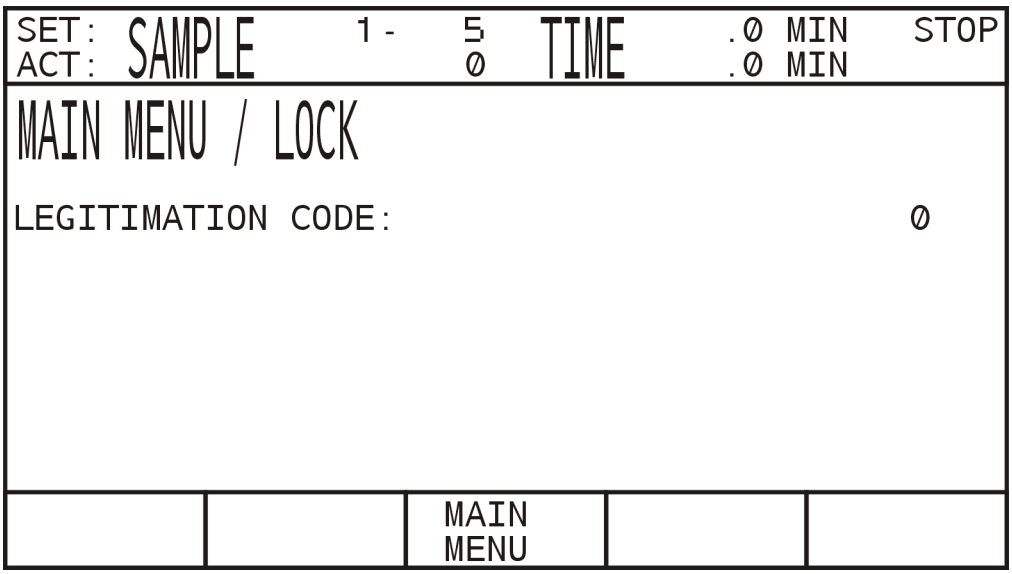
| | |
|---|---|
| n **STATUS** | Display the Status Screen (also key [**F1**]). |
| n **PRIORITY SAMPLE** | Display the Priority Sample Menu (also key [**F5**]). |
| n **SAMPLE SEQUENCE** | Show the Sample Sequence Menu. |
| n **ANALYSIS PROGRAM** | Show the Analysis Program Menu. |
| n **WASH PROGRAM** | Show the Wash Program Menu. |
| n **INSTRUMENT CONFIGURATION** | Show the Instrument Configuration Menu |
| n **DERIVATISATION MENU** | Show the Derivatisation Menu (optional) |

# FUNCTION KEYS

| Key | Function | Description |
|---|---|---|
| **[F1]** | **STATUS** | Show the Status Screen. |
| **[F3]** | **MENU LOCK** | Show the Main Lock / Unlock Menu. |
| **[F5]** | **PRIO SAMPLE** | Show the Priority Sample Menu. |

## 7.1.1 MAIN MENU: LOCK

All menus and settings can be protected against manipulations by illegitimated operators.




**Picture 15: Menu Lock**

## DESCRIPTION

To lock all menus, enter the MENU LOCK menu by pressing [**F3**] in the MAIN MENU and entering the legitimation code using the numeric keys. When the code is confirmed with the [**ENTER**] key, all menus and settings are locked until the system is unlocked again.

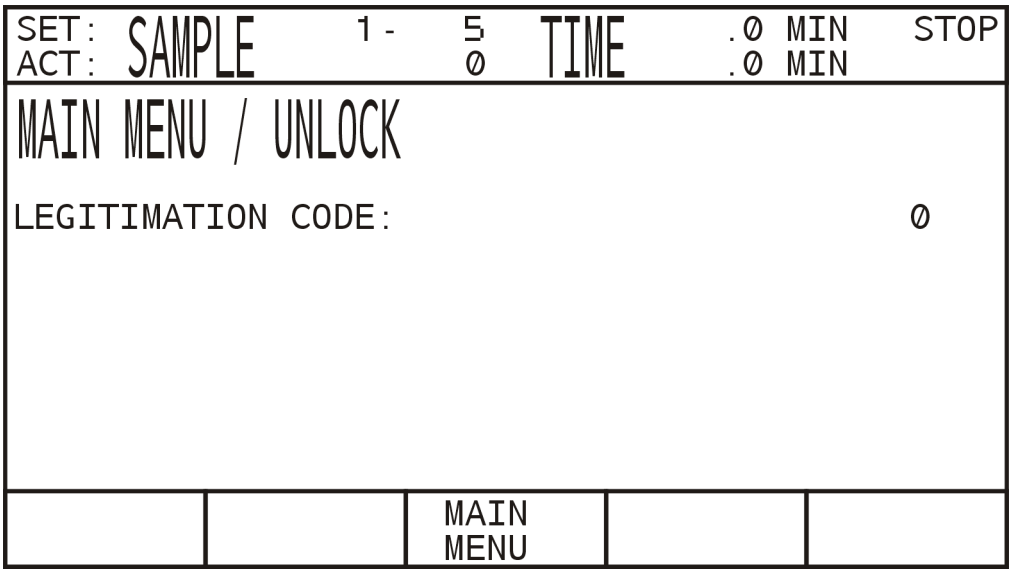
The Legitimation code is: **23456**.

## FUNCTION KEYS

| Key | Function | Description |
|---|---|---|
| **[F3]** | **MAIN MENU** | Return to the Main Menu. |

## 7.1.2 MAIN MENU: UNLOCK

When the system is locked, you first have to unlock all menus before you can make any changes to the system's parameters.




**Picture 16: Menu Unlock**

## DESCRIPTION

To unlock all menus, enter the MENU UNLOCK menu by pressing [**F3**] in the MAIN MENU and entering the legitimation code using the numeric keys. When the code is confirmed with the [**ENTER**] key, all menus and settings are unlocked again.

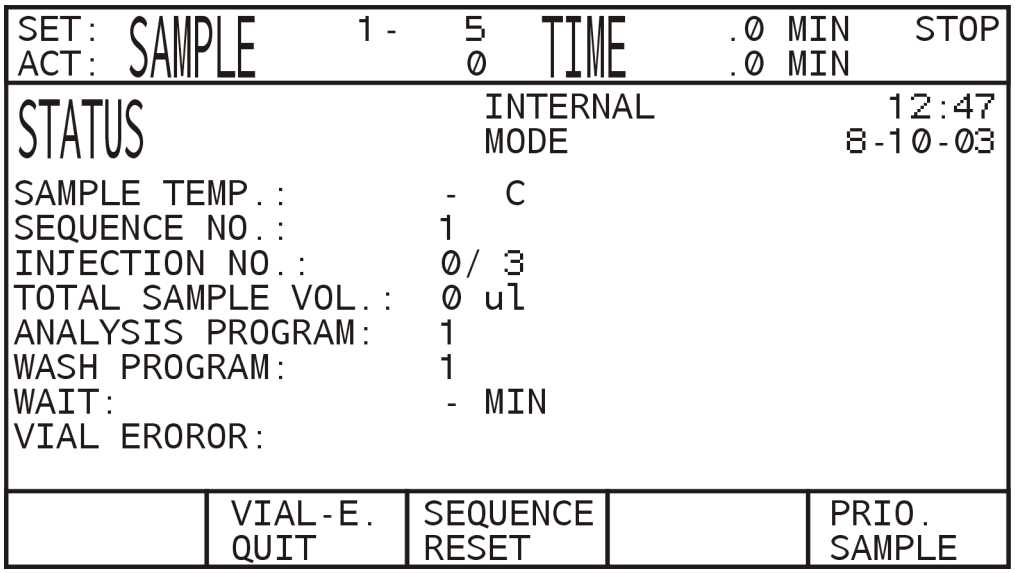
The Legitimation code is: **23456**.

## FUNCTION KEYS

| Key | Function | Description |
|---|---|---|
| **[F3]** | **MAIN MENU** | Return to the Main Menu. |

# 7.2 STATUS SCREEN

After pressing the [**F1**] or [**0**] key in the MAIN MENU, the Status of the system is shown on the screen. All parameters important for the analysis are shown. We recommend that this menu is displayed during operation.




**Picture 17: Status Screen**

## DESCRIPTION

The STATUS SCREEN displays the following information:

| | |
|---|---|
| n **SAMPLE TEMP** | If the cooling option is available, this field shows the current sample temperature. |
| n **SEQUENCE NO** | This field displays the current sample sequence |
| n **INJECTION NO** | This field shows the Current Injection and the total number of injections for the current vial. |
| n **TOTAL SAMPLE VOL** | This field shows the total sample volume taken by the instrument. |
| n **ANALYSIS PROGRAM** | Here you can see the number of the Analysis Program used for the current sample. |
| n **WASH PROGRAM** | This field displays the number of the used Wash Program. |
| n **WAIT** | In this field you can see the Wait Time countdown, if you have selected a Wait Time in the Instrument Configuration Menu. |
| n **VIAL ERROR** | In case of an Vial Error, this field displays a row of numbers; each the vial number where an Vial Error occurred. You can delete each single entry by pressing the [F2] key. This is |

necessary to restart a sequence, as the instrument cannot be started as long as there are any errors displayed in this line.

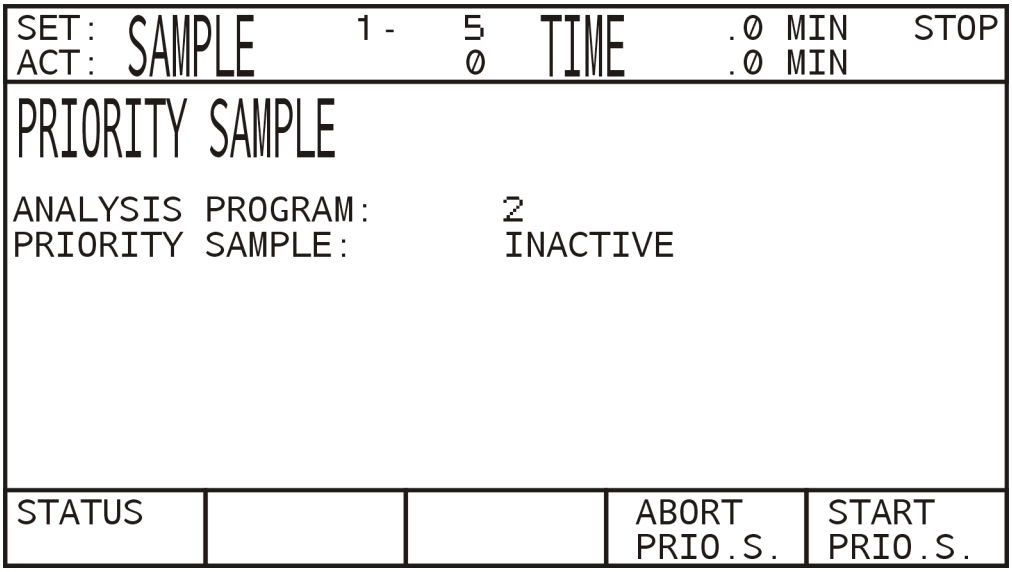
# FUNCTION KEYS

| Key | Function | Description |
|---|---|---|
| **[F2]** | **VIAL-E. QUIT** | Clear any Vial Errors. This has to be done before you can start a Sequence and any Vial Errors are displayed. |
| **[F3]** | **SEQUENCE RESET** | Reset the Sequence to start at the first vial/first injection again. |
| **[F5]** | **PRIO SAMPLE** | Show the Priority Sample Menu |

# 7.3 PRIORITY SAMPLE MENU

The priority sample screen is selected by **Main Menu and (1)** or directly by selecting the **Function Key F5** (Priority sample). When activating the priority sample **(F5)** the sample placed in vial position **120** will be injected immediately after the actual analysis is finished, without stopping the current run. For processing the priority sample, a different analysis program no. can be used.

If requested, the vial position of the priority sample can be changed in the submenu. This function is not possible during serial operation.




**Picture 18: Priority Sample Menu**

## DESCRIPTION

The Priority Sample Menu displays the following information:

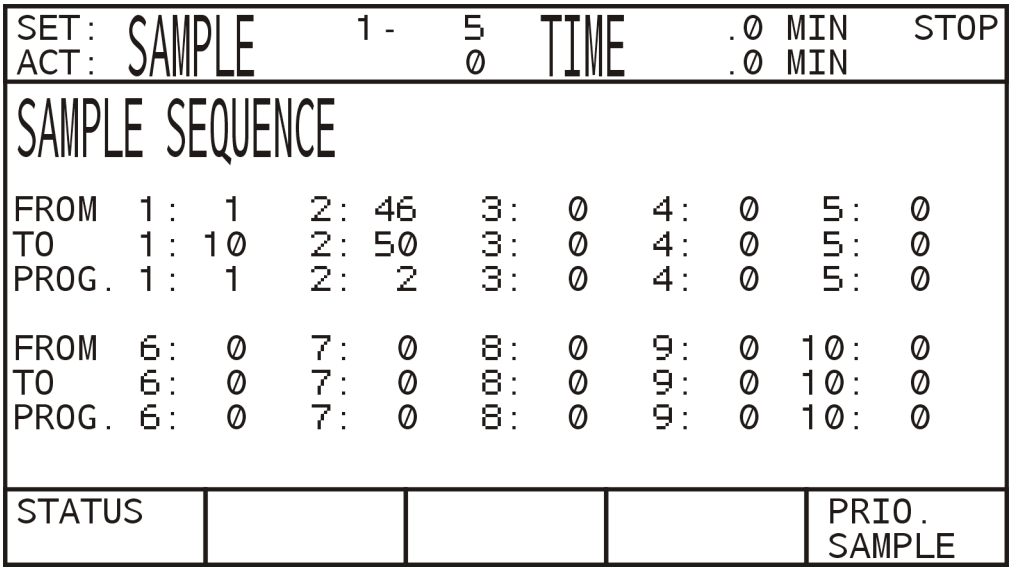
| | |
|---|---|
| n **ANALYSIS PROGRAM** | Enter the number of the Analysis Program to be used for the Priority Sample. |
| n **PRIORITY SAMPLE** | In this field you can see if the Priority Sample is Inactive or Active. The information is also displayed in the Status Bar right next to “SAMPLE” |

## FUNCTION KEYS

| Key | Function | Description |
|---|---|---|
| **[F1]** | **STATUS** | return to Status Screen |
| **[F4]** | **ABORT PRIO. S.** | Abort the Priority Sample Run |
| **[F5]** | **START PRIO. S.** | Start the Priority Sample Run |

# 7.4 SAMPLE SEQUENCE MENU

From the **Main Menu and (2)** the sample sequence screen is selected. This screen combines the samples (vial: from to) with the analysis program.




**Picture 19: Sample Sequence Menu**

## DESCRIPTION

The Sample Sequence Menu displays the following information:

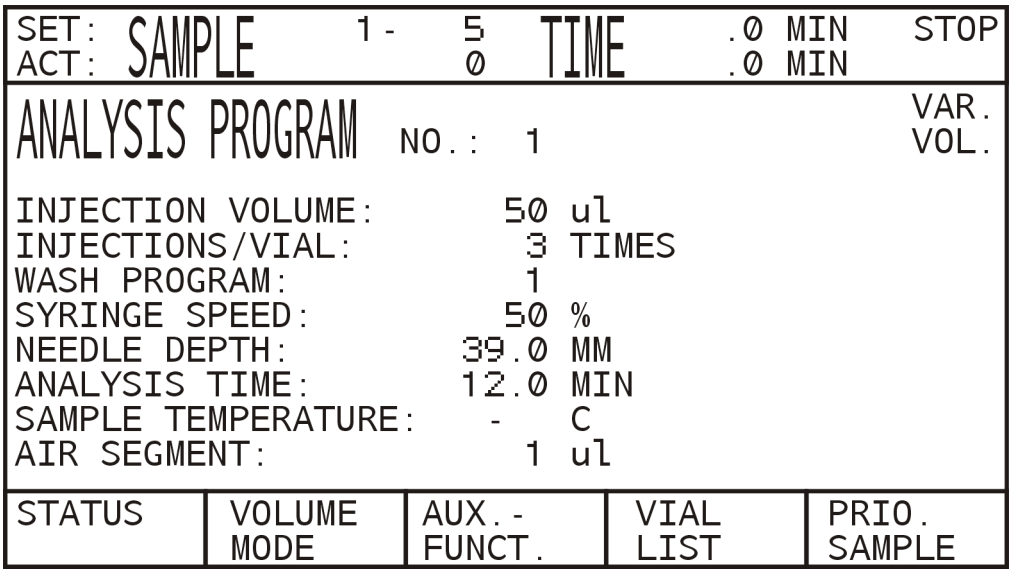
| | |
|---|---|
| n **FROM** | Enter the first vial of the sequence step |
| n **TO** | Enter the last vial of the sequence step. |
| n **PROG** | Enter the Number of the Analysis Program to be used for this sequence step. |

## FUNCTION KEYS

| Key | Function | Description |
|---|---|---|
| **[F1]** | **STATUS** | return to Status Screen |
| **[F5]** | **PRIO. SAMPLE** | Show Priority Sample Menu |

# 7.5 ANALYSIS PROGRAM MENU

The analysis program is selected by using the Main Menu and key [**3**]. In this menu, all for the analysis important parameters are entered. It is possible to store a multitude of different programs which can be individually selected by entering the number of the program.




**Picture 20: Analysis Program Menu**

## DESCRIPTION

The Analysis Program Menu displays the following information:

| | |
|---|---|
| n **NO** | The number of the analysis program to be edited |
| n **INJECTION VOLUME** | This value is dependent on the currently selected volume mode. The mode can be changed with the [**F2**] function key and is displayed on the right upper corner of the content area.<br>**Variable Volume:** Enter the injection volume in µl<br>**Fix Volume:** Enter the number of times the sample loop should be overfilled; e.g. 3 times<br>*Note that the variable volume mode is only available with the variable volume injection option.* |
| n **INJECTIONS/VIAL** | Enter the number of injections to be performed for each vial. |
| n **WASH PROGRAM** | Enter the number of the wash program to be used with this analysis program. |
| n **SYRINGE SPEED** | Here you can select a different speed for the syringe movement. Besides the default value of 50% you can select 100% and 33 %. |

| | |
|---|---|
| n **NEEDLE DEPTH** | This value specifies the depth of the injection needle movement. This value have to be adjusted to specific vials (e.g. Eppendorf vials use a Needle Depth of 39 mm) |
| n **ANALYSIS TIME** | This is the runtime for this analysis program. The next sample injection will start after this time has elapsed. |
| n **SAMPLE TEMPERATURE** | Here you can enter the temperature of the sample racks. *Note that this feature is only available with the cooling option.* |
| n **AIR SEGMENT** | Here you can enter the size of the air segment between the taken sample and the eluent. This feature prevents the mixture of eluent and sample which leads to an inaccurate sample injection. |

# NOTES

If you control the S 5200 Autosampler via RS-232, Analysis Program #1 will be used. Make sure that all parameters of program #1 are valid for your method.

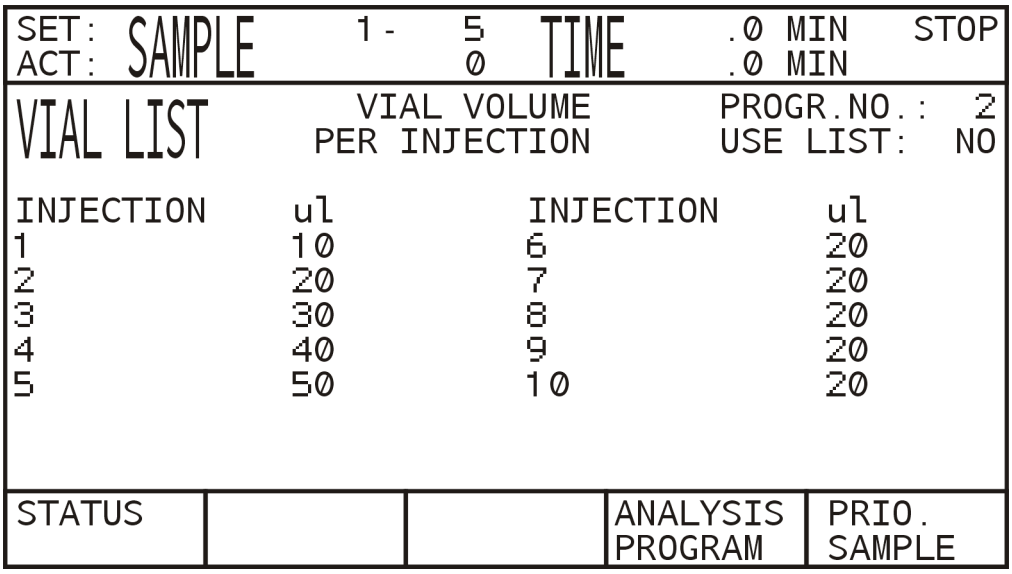
# FUNCTION KEYS

| Key | Function | Description |
|---|---|---|
| **[F1]** | **STATUS** | return to Status Screen |
| **[F2]** | **VOLUME MODE** | Switch between fix and variable volume mode. *Note that the variable volume mode is only available with the variable volume injection option.* |
| **[F3]** | **AUX.-FUNCT.** | Switch to the Auxiliary Functions Menu |
| **[F4]** | **VIAL LIST** | Switch to the Vial List Menu |
| **[F5]** | **PRIO. SAMPLE** | Show Priority Sample Menu |

# 7.6 VIAL LIST MENU

This submenu can be selected by pressing the [**F4**]. With variable volume injection operation, it is possible to program different sample volumes, taken from the same sample vial.

With this function, automatic calibration functions can be programmed without additional dilution.




**Picture 21: Vial List Menu**

## DESCRIPTION

The Vial List Menu displays the following information:

| | |
|---|---|
| n **PROG. NO.** | The number of the vial list to be edited and/or used |
| n **USE LIST** | Here you can select if this list should be used in the analysis or not. |
| n **INJECTION** | This column specifies the respective injection number |
| n **µL** | Enter the desired injection volume in this column |

## FUNCTION KEYS

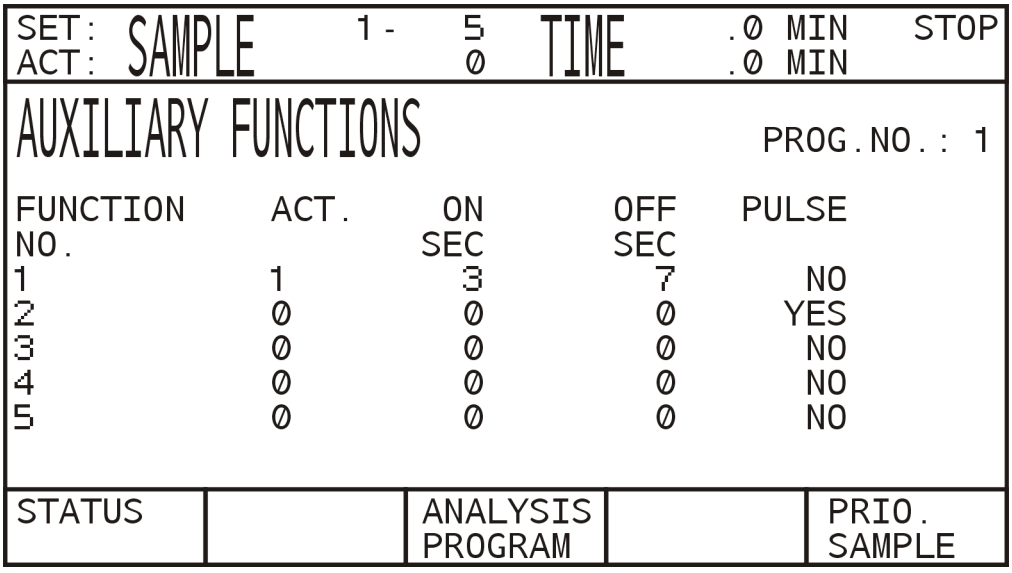
| Key | Function | Description |
|---|---|---|
| **[F1]** | **STATUS** | return to Status Screen |
| **[F4]** | **ANALYSIS PROGRAM** | Return to the Analysis Program Menu |
| **[F5]** | **PRIO. SAMPLE** | Show Priority Sample Menu |

# 7.7 AUXILIARY FUNCTIONS MENU

The auxiliary function program is selected from the ANALYSIS PROGRAM by using the [**F3**] key.

Five relay functions can be programmed which will activate external functions or pulses. With the [**CURSOR**] keys the positions for entering the values can be selected. The values are entered with the numeric keys or with the [**SELECT**] key. All time values are relative to the time of injection; with the injection of a sample, the time counter starts at 0.

For the pulse transfer, a 15-pin connector is installed at the rear side of the instrument (AUX. FUNCTION). The relays can be programmed for setting defined signals (on/off) or automatic switching after a certain pulse duration, entered in the Instrument Configuration Menu.




**Picture 22: Auxiliary Functions Menu**

## DESCRIPTION

The Vial List Menu displays the following information:

| | |
|---|---|
| n **PROG. NO.** | The number of the Analysis Program to be edited. |
| n **FUNCTION NO.** | The number of the Output relay |
| n **ACT.** | Display of the actual status of the relay; 0 = OFF; 1 = ON |
| n **ON SEC** | Enter the time when the relay is switched on. |
| n **OFF SEC** | Enter the time when the relay is switched off. |
| n **PULSE** | Select if this output should use a pulse or a static signal.<br>**PULSE** Signal is switched on for a time period specified in the INSTRUMENT CONFIGURATION MENU. After this period the relay is |

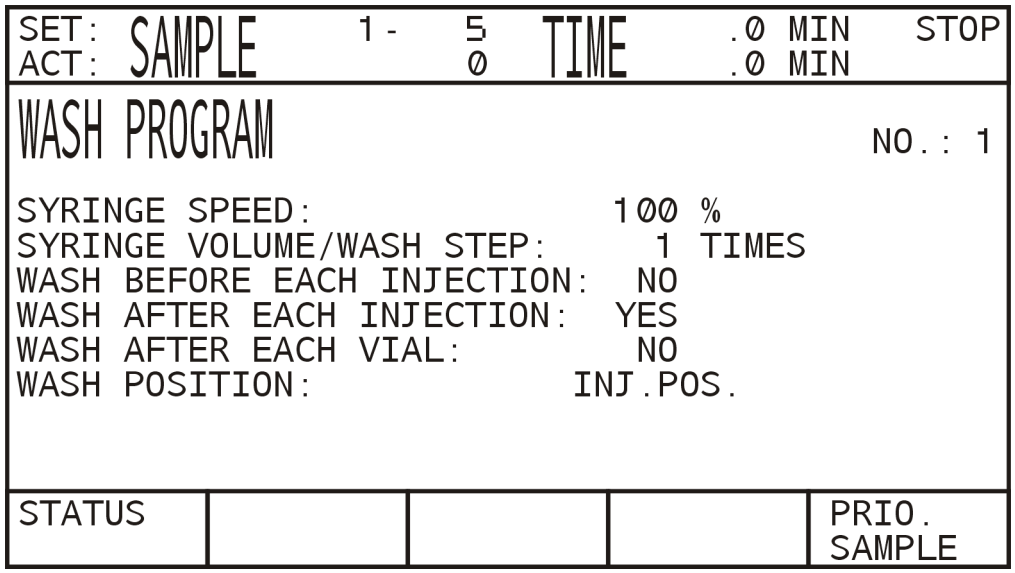
| | |
|---|---|
| | automatically switched off. |
| **STATIC** | When PULSE = NO, the output signal must be manually switched on and off. |

# FUNCTION KEYS

| Key | Function | Description |
|---|---|---|
| **[F1]** | **STATUS** | Return to the Status Screen |
| **[F3]** | **ANALYSIS PROGRAM** | Return to the Analysis Program Menu |
| **[F5]** | **PRIO. SAMPLE** | Show Priority Sample Menu |

# 7.8 WASH PROGRAM MENU

The wash program is selected by using the **Main Menu and (4)**. It controls the wash functions before and/or after the injection. Several wash steps can be combined. The wash program is activated through the analysis program.




**Picture 23: Wash Program Menu**

## DESCRIPTION

The Wash Program Menu displays the following information:

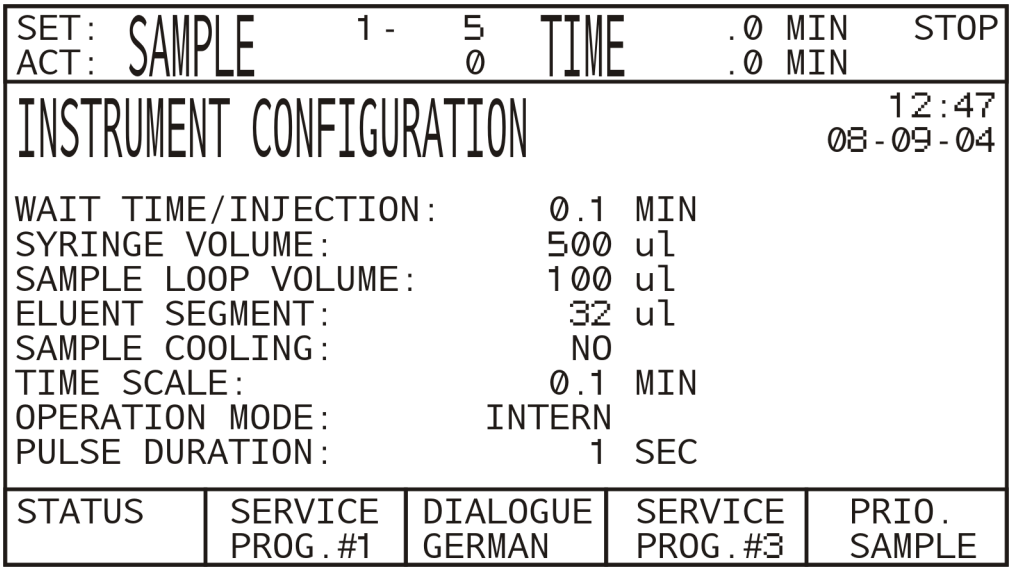
| | |
|---|---|
| n **NO.** | The number of the wash program to be edited |
| n **SYRINGE SPEED** | Here you can change the speed of the syringe drive. Possible values are 100%, 50%, and 33% |
| n **SYRINGE VOLUME/ WASH STEP** | Specify the number of times the complete syringe volume should be used for each wash step. |
| n **WASH BEFORE EACH INJECTION** | Select YES, when you want to wash before each injection. |
| n **WASH AFTER EACH INJECTION** | Select YES, when you want to wash before each injection. |
| n **WASH AFTER EACH VIAL** | Select YES, when you want to wash after each vial. |
| n **WASH POSITION** | Select at which position the wash step should be performed. You can either select the Injection Port (*Inj.Pos.*) or the default Washing Port (*Default*). |

# FUNCTION KEYS

| Key | Function | Description |
|---|---|---|
| **[F1]** | **STATUS** | return to Status Screen |
| **[F5]** | **PRIO. SAMPLE** | Show Priority Sample Menu |

# 7.9 INSTRUMENT CONFIGURATION MENU

The instrument configuration program is selected by using the MAIN MENU and key [**5**]. The parameters defined for the operation are displayed and can be adjusted.




**Picture 24: Instrument Configuration Menu**

## DESCRIPTION

The Instrument Configuration Menu displays the following information:

| | |
|---|---|
| n **TIME / DATE** | At the upper right corner of the content area, the individual time and date positions can be changed by using the [CURSOR] keys. After entering the new time/date, the [ENTER] key has to be pressed. |
| n **WAIT TIME / INJECTION** | Here you can enter a wait time before each injection. If a wait time is entered, the Aux. Functions 1 and 2 are occupied and cannot be used for custom purposes. |
| n **SYRINGE VOLUME** | This is the volume [µl] of the syringe used. Only change this value if you install a new syringe. |
| n **SAMPLE LOOP** | This is the volume [µl] of the installed sample loop. You can change this value if you install an other sample loop. |
| n **ELUENT SEGMENT** | The Eluent Segment is a specific volume of wash solution for delivering the sample into the sample loop. |
| | **Fix Volume:** 5-15 µl |

| | |
|---|---|
| | **Variable Volume:** ~35-40 µl |
| n **SAMPLE COOLING** | If a cooling/heating unit (optional) is installed, the requested temperature can be entered here; otherwise NO is displayed. |
| n **TIME SCALE** | Use the [**SELECT**] key to define the time scale of the instrument; available options are: seconds, 0.1 min. or 1 min. |
| n **OPERATION MODE** | Select the appropriate operation mode here:<br>**INTERN:** The instrument is controlled internally by pressing [**START**] and [**STOP**]<br>**EXTERN:** The instrument is controlled externally via TTL start signal<br>**SERIAL:** The instrument is controlled via RS-232 serial interface. This mode is used when the S 5200 is controlled by a data system (e.g. ChromStar) |
| n **PULSE DURATION** | Here you can enter the duration of the TTL relay pulses, like the START or INJECT function. |

# FUNCTION KEYS

| Key | Function | Description |
|---|---|---|
| **[F1]** | **STATUS** | return to Status Screen |
| **[F2]** | **SERVICE PROG.#1** | Switch to Service Program #1 Menu |
| **[F3]** | **DIALOGUE GERMAN** | Switch between English and German language |
| **[F4]** | **SERVICE PROG.#3** | Switch to Service Program #3 Menu |
| **[F5]** | **PRIO. SAMPLE** | Show Priority Sample Menu |

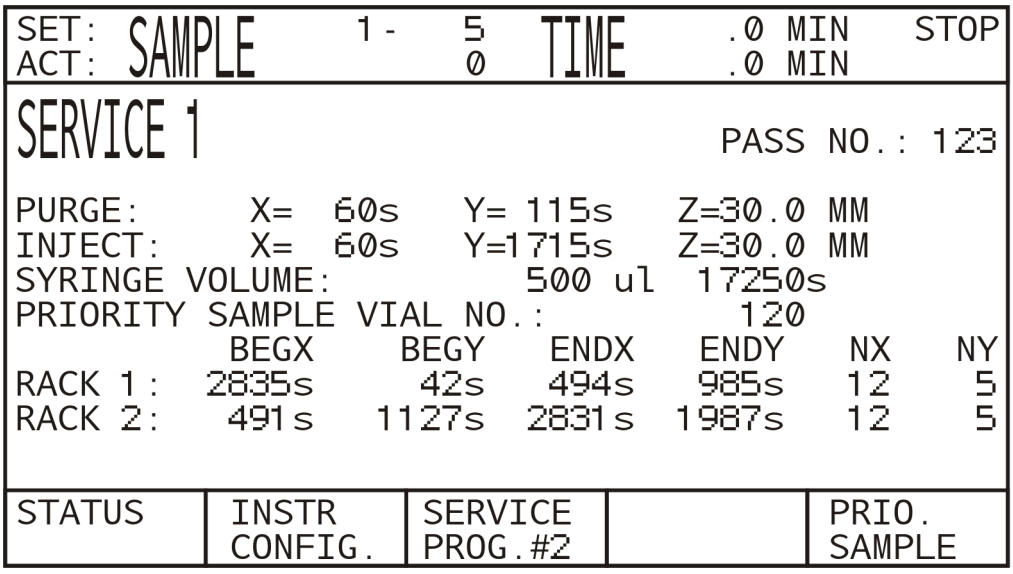
## 7.9.1 SERVICE PROGRAM #1

The service program is displayed by selecting the **Instrument Configuration Menu** and pressing the **Function Key F2**.

In this program the standard position settings are displayed. When using different sample racks, the values have to be adjusted in this program. For different operation, various racks are available:

- § analytical version: racks for 120 vials with 1.5 ml volume
- § preparative version: racks for 48 vials with 5 ml volume
- § micro version: sliding unit for fixing micro titer plates

**NOTE**: If incorrect values are entered, the needle can be damaged! Before changing the values, they should be documented. If there are any problems or questions concerning the programming, please get in contact with the manufacturer.




**Picture 25: Service Menu #1**

## DESCRIPTION

The Service Menu #1 displays the following information:

| | |
|---|---|
| n **PASS NO.** | Enter the legitimation code (“123”) here. |
| n **PURGE** | X/Y/Z-coordinates of the wash port |
| n **INJECT** | X/Y/Z-coordinates of the injection port |
| n **SYRINGE VOLUME** | Enter Number of steps for the entire syringe volume (default = 17250) |

| | |
|---|---|
| n **PRIORITY SAMPLE VIAL NO.** | Enter the Vial Position of the priority sample (default = 120) |
| n **RACK 1** | This row of coordinates is valid for Rack #1 (left) |
| n **RACK 2** | This row of coordinates is valid for Rack #2 (right) |
| n **BEG X** | X-position of the first vial in this rack |
| n **BEG Y** | Y-position of the first vial in this rack |
| n **END X** | X-position of the last vial in this rack |
| n **END Y** | Y-position of the last vial in this rack |
| n **NX** | Number of vials in X-Axis of this rack |
| n **NY** | Number of vials in Y-Axis of this rack |

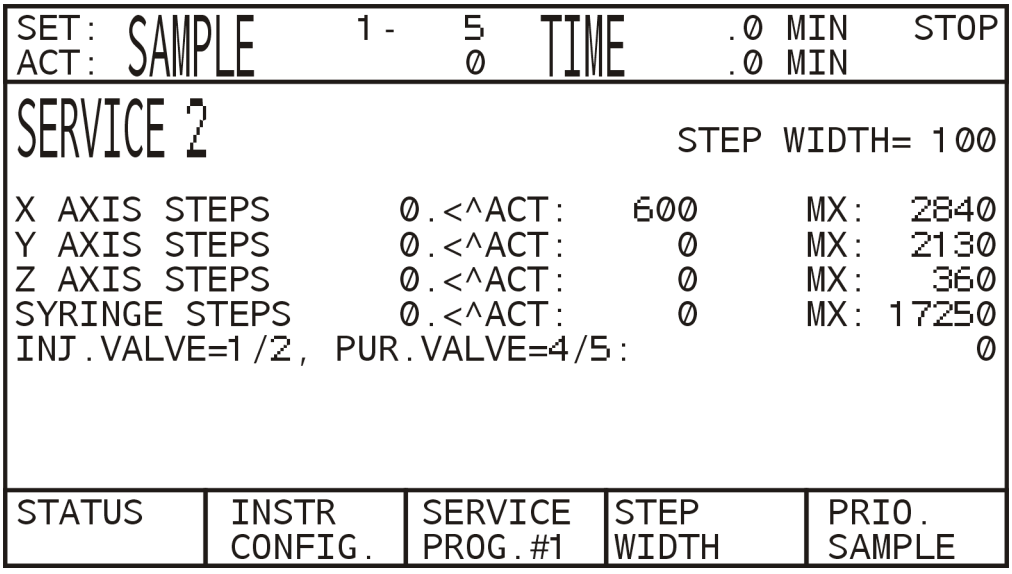
# FUNCTION KEYS

| Key | Function | Description |
|---|---|---|
| **[F1]** | **STATUS** | return to Status Screen |
| **[F2]** | **INSTR. CONFIG.** | Return to INSTRUMENT CONFIGURATION Menu |
| **[F3]** | **SERVICE PROG.#2** | Show SERVICE PROGRAM #2 Menu |
| **[F5]** | **PRIO. SAMPLE** | Show PRIORITY SAMPLE Menu |

## 7.9.2 SERVICE PROGRAM #2

The service program is displayed by selecting the **Service Program # 1** and pressing the **Function Key F3**. **Note:** The program can be only entered, if the legitimation code (PASS NO.) was set in the Service Program #1.

In this submenu, the exact value settings of the needle's position co-ordinates can be determined. For this, the needle is manually moved with the cursor keys to the single positions and the co-ordinates displayed have to be documented.




**Picture 26: Service Menu #2**

## DESCRIPTION

The Service Menu #2 displays the following information:

| | |
|---|---|
| n **STEP WIDTH** | Display of the currently selected step width. This is the amount of step by which the selected axis will be moved. |
| n **X AXIS STEPS** | Current position (in steps) of the X-Axis |
| n **Y AXIS STEPS** | Current position (in steps) of the Y-Axis |
| n **Z AXIS STEPS** | Current position (in steps) of the Z-Axis |
| n **MX** | Max. number of steps possible for this axis |
| n **SYRINGE STEPS** | Current position (in steps) of the Syringe-Axis |
| n **INJ. VALVE** | You can manually move the injection valve with the keys [**1**] and [**2**]; press either key and confirm with [**ENTER**]. The injection valve can then be moved to the selected position by pressing the [**SEL_UP**] or [**SEL_DOWN**] key. |

| | |
|---|---|
| n **PURGE VALVE** | You can manually move the purge valve with the keys [**4**] and [**5**]; press either key and confirm with [**ENTER**]. The injection valve can then be moved to the selected position by pressing the [**SEL_UP**] or [**SEL_DOWN**] key. |

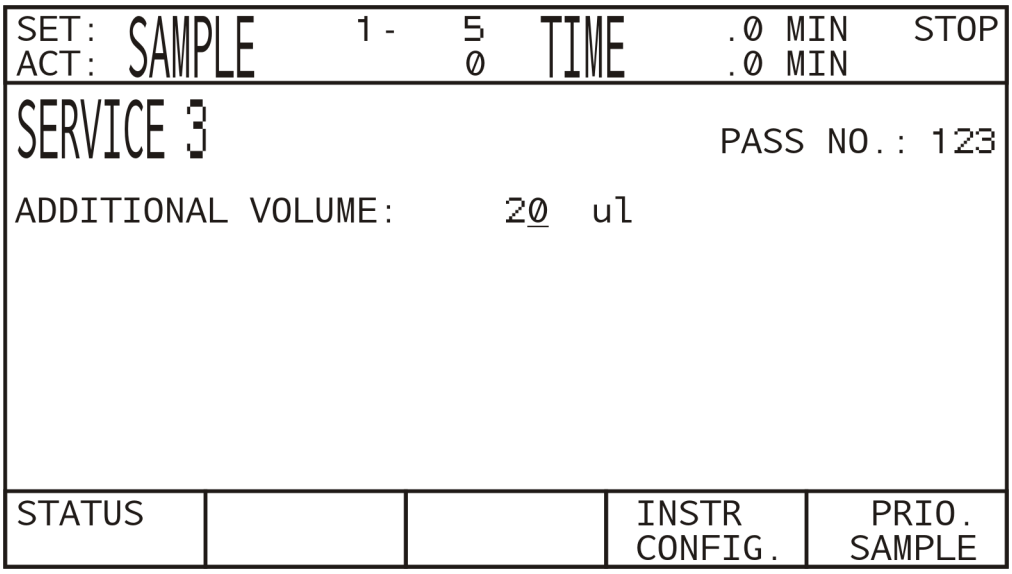
# FUNCTION KEYS

| Key | Function | Description |
|---|---|---|
| **[F1]** | **STATUS** | return to Status Screen |
| **[F2]** | **INSTR. CONFIG.** | Return to INSTRUMENT CONFIGURATION Menu |
| **[F3]** | **SERVICE PROG.#1** | Show SERVICE PROGRAM #1 Menu |
| **[F4]** | **STEP WIDTH** | The step width for the axis movement can be set with this key |
| **[F5]** | **PRIO. SAMPLE** | Show PRIORITY SAMPLE Menu |

## 7.9.3 SERVICE PROGRAM #3

The service program is displayed by selecting the **Service Program # 1** and pressing the **Function Key F4**. **Note:** The program can be only entered, if the legitimation code (PASS NO.) was set in the Service Program #1.

In this submenu, the Additional Volume for the sample injection can be set. Normally, stay with the default value of 20 µl.




**Picture 27: Service Menu #3**

## DESCRIPTION

The Service Menu #2 displays the following information:

| | |
|---|---|
| n **ADDITIONAL VOLUME** | Display of the currently set Additional Volume. This is the volume which is taken from a sample vial in addition to the programmed sample volume. It is used to exactly move the sample to the beginning of the sample loop. |

## FUNCTION KEYS

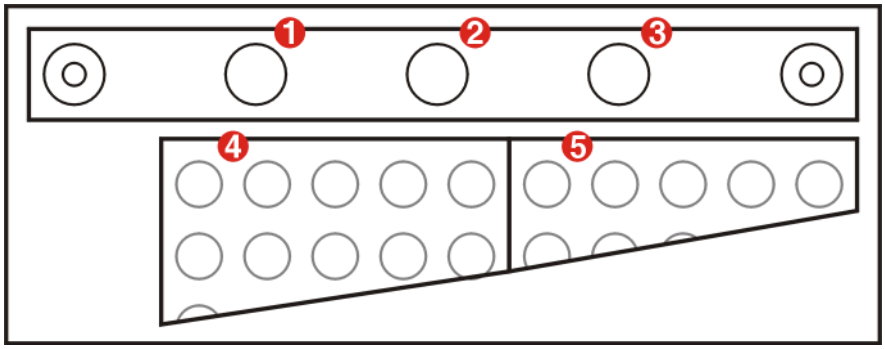
| Key | Function | Description |
|---|---|---|
| **[F1]** | **STATUS** | return to Status Screen |
| **[F4]** | **INSTR. CONFIG.** | Return to INSTRUMENT CONFIGURATION Menu |
| **[F5]** | **PRIO. SAMPLE** | Show PRIORITY SAMPLE Menu |

# 8. DERIVATISATION

The derivatisation program is selected by using the MAIN MENU and key [**6**] or by using the cursor, if the derivatisation option is available. Otherwise, the DERIVATISATION MENU is missing altogether.

The Autosampler is equipped with an automatic pre-column derivatisation/automatic sample dilution mode, i.e. up to three different derivatisation reagents and the wash solution can be automatically added to the sample. Each dosage added to the sample. Mixing cycle and reaction time is variable.

The vials with the samples have to be positioned in the right sample rack (61-120). Empty sample vials will be positioned in the left sample rack (1-60); they are used as mixing vials, i.e. each sample vial has a defined mixing vials at the corresponding position. The derivatisation vials (5 ml) are positioned behind the sample racks.




**Picture 28: Derivatisation Vial Positions**

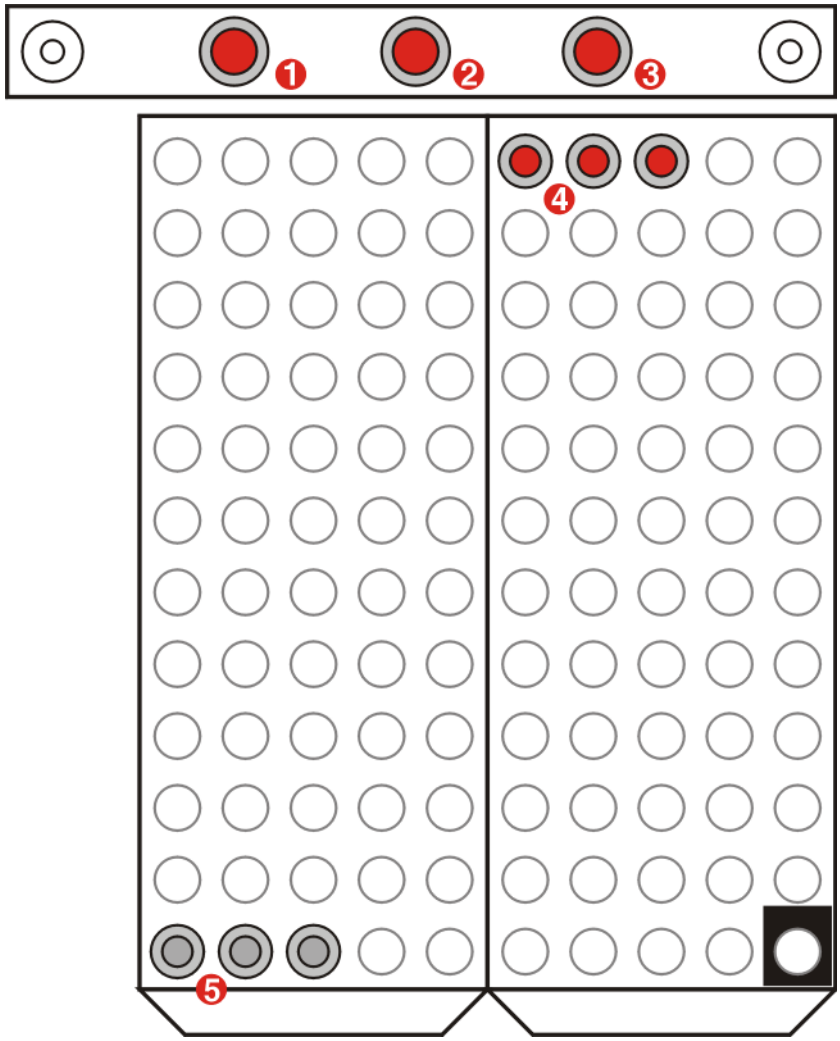
1 Derivatisation Vial A
2 Derivatisation Vial B
3 Derivatisation Vial C
4 Mixing Vials Rack
5 Sample Vials Rack

## 8.1 DERIVATISATION EXAMPLE

The following example assumes the following procedure:

The sample has to be mixed with 3 different Reagents A, B and C. The sample should be mixed with 10 µl Reagent A, 20 µl Reagent B and 10 µl Reagent C. The reaction time for Reagent A is 10 seconds, for Reagent B 10 seconds and for Reagent C 5 seconds. The total Derivatisation Time is 60 seconds. Finally, the sample should be diluted by 50%.

The use now prepares three 5ml vials with the Reagents A, B and C and places them in the Derivatisation Vial places. Then he places three empty vials in the left sample rack at positions 1 – 3. Finally, he places his 3 samples in the right sample rack at positions 61 – 63. The positions of the individual vials are shown on the diagram below.




**Picture 29: Derivatisation Example**

1 Reagent A (5 ml Vial)
2 Reagent B (5 ml Vial)
3 Reagent C (5 ml Vial)
4 Sample Vials (#61-63)
5 Empty Vials (#1-3)

After the placement of the vials the user has to setup the instrument's programs as outlined below.

## ANALYSIS PROGRAM

In the ANALYSIS PROGRAM menu the user selects program number 4 for storing it. Then he enters all parameters according to his samples' method:

- Volume Mode: Variable
- Injection Volume: 10 µl
- Injections / Vial: 3
- Time: 20 minutes
- Wash Program: 1

## SAMPLE SEQUENCE

In the SAMPLE SEQUENCE menu the user specifies the sequence according to the diagram above:

- From: 61
- To: 63
- Prog.: 4

## DERIVATISATION PROGRAM

The last step the user has to conduct is editing the Derivatisation Program. In the DERIVATISATION PROGRAM menu he enters the following:

- No.: 1
- Sample: 30 µl
- Reag. A: 10 µl
- Mixing: 4 x
- React. Time: 10 sec.
- Reag. B: 20 µl
- Mixing: 5 x
- React. Time: 10 sec.
- Reag. C: 10 µl
- Mixing: 4 x
- React. Time: 5 sec.
- Dilution: 70 µl (≅ 50% dilution; 70 µl total volume of Sample + Reagents)
- Wash after each Injection: YES
- Air Segment: 3 µl

# 9. OPERATION

## 9.1 SETUP PROGRAMS

Hereby, stepwise procedures for various often needed operations are described. Please note, these are only suggestions, no defined instruction procedures.

### 9.1.1 ANALYSIS PROGRAM

First, you have to enter an Analysis Program to be used for the sample sequence.

- press [**MENU**] key.
- move the cursor to the Analysis Program and press [**ENTER**], or directly enter the menu with the [**3**] key.
- move the cursor to the NO. field and enter the program number under which this Analysis Program will be stored (e.g. “3”).
- move the cursor to the INJECTION VOLUME field. Enter the injection volume (variable injection volume) or the multiplier by which the sample loop should be over-filled (fix volume injection).
- with multiple injection, enter the injections to be done per vial.
- move the cursor to wash program and enter the no. of the wash program to be used (e.g. “3”).
- move the cursor to the SYRINGE SPEED field and select 50 %.
- move to the NEEDLE DEPTH field and check if the needle depth is appropriate for the used vials (2ml conical plastic vials: 39mm, 1.5ml glass vials: 30mm).
- move the cursor to the ANALYSIS TIME field and enter the time duration for a single analysis.
- *with upgrade option cooling/heating*: enter the temperature (in case, the heating/cooling option should be switched off: enter “0”).
- move the cursor to the AIR SEGMENT field and enter a suitable value (recommended value: 3 µl).
- all entered values are stored under the given program no.

### 9.1.2 WASH PROGRAM

- press [**MENU**] key.
- move the cursor to the WASH PROGRAM MENU and press [**ENTER**], or directly enter the menu with the [**4**] key.

- with cursor to No., enter the program no. under which this program should be stored
- recommendation for standard operations: 100 % syringe speed, 1 wash step, wash after each injection, wash position: inj. pos.
- all entered values are stored under the given program no.

### 9.1.3 SAMPLE SEQUENCE

- press [**MENU**] key.
- move the cursor to the SAMPLE SEQUENCE MENU and press [**ENTER**], or directly enter the menu with key [**2**].
- move the cursor to the FROM field and enter the number of the first vial of the sequence (e.g. “1”).
- move the cursor to the TO field and enter the number of the last vial of the sequence (e.g. “10”).
- Move the cursor to the PROG field and enter the number of the analysis program to be used for this sequence (e.g. “3” for the number of the analysis program you’ve prepared above).

## 9.2 RUN THE INSTRUMENT

### 9.2.1 STARTING AN ANALYSIS SEQUENCE

- check the sample racks (positioned correctly and if the vials are placed correctly and filled with sample).
- press the [**STATUS**] key.
- before starting the sequence, press [**F3**] (SEQUENCE RESET). In case of any error messages, these have to be deleted with the [**STOP**] or [**F2**] (Vial Error) key.
- check the operation mode (INTERN, EXTERN). Adjustments can be done in the CONFIGURATION MENU ([**MENU**] [**5**]).
- press the [**START**] key.
- during the sample processing, changes can be done to all the submenus which will be activated in the next injection.

### 9.2.2 STOPPING THE ANALYSIS

In case an error was made (e.g. mistake in filling the vial) which is detected after the analysis has been started, the analysis process can be stopped by pressing the [**STOP**] key. This will stop the current time counter, the functions however will remain activated. In the top right

corner 'HOLD' will be displayed. Now, the vial can be placed in the correct position and after pressing the [**START**] key, the operation is activated again.

In case, a serious problem appears and the analysis sequence should be totally stopped, the [**STOP**] key has to be pressed once again.

Select in the MAIN MENU the STATUS display and by pressing [**F3**] (SEQUENCE RESET), the sequence will start again with the first sample. The [**START**] key has to be pushed to start the processing.

## 9.2.3 EXTERNAL CONTROL

- press the [**MENU**] key .
- move the cursor to the INSTRUMENT CONFIGURATION MENU and press [**ENTER**], or directly enter the menu with key [**5**].
- Move the cursor down to the OPERATION MODE field. Using the [**SEL_UP**] and [**SEL_DOWN**] keys will change between the different modes. Select the EXTERN mode. Now the instrument is controlled via digital signals of an external controller.

## 9.2.4 SERIAL OPERATION

When selecting the serial mode, the autosampler is controlled with the help of an external software (e.g. ChromStar).

- press the [**MENU**] key .
- move the cursor to the INSTRUMENT CONFIGURATION MENU and press [**ENTER**], or directly enter the menu with key [**5**].
- move the cursor down to the OPERATION MODE field. Using the [**SEL_UP**] and [**SEL_DOWN**] keys will change between the different modes. Select the SERIAL mode. Now the instrument is controlled by RS-232 serial commands.
- press the [**F1**] key to enter the STATUS SCREEN ! This is very important, as the S5200 has to be in the STATUS SCREEN when serial communication from a PC is started.
- now the data system can control the autosampler.

## 9.2.5 AUXILIARY FUNCTIONS

The S5200 features an auxiliary port with 5 separate analog outputs. These can be used to control external instruments, like motor valves. The individual output (No. 1 to 5) can be programmed in the AUXILIARY FUNCTIONS Menu (7.7).

**NOTE: Auxiliary Function No.1 is pre-defined when the Wait Time is activated (see SPECIAL FUNCTION below) !**

# SPECIAL FUNCTION

When a Wait Time >0 is programmed, Auxiliary Function No. 1 (AF1) is used in a certain way. The AF1 signal is activated as soon as the **[START]** key is pressed; this signal can be used to start an external instrument, like a gradient pump. When a Wait Time of 0 is programmed, AF1 is dormant and can be programmed otherwise in the AUXILIARY FUNCTIONS Menu (7.7).

# 9.3 ERROR MESSAGES

In case of an error, a short error code is shown in the top right corner of the status area.

| Display | Error | Description | Solution |
|---|---|---|---|
| **LOGICAL / OPERATIONAL ERRORS** | | | |
| **ERR??** | "Syringe Volume Error" / "Undefined Error" | Syringe volume was exceeded, or other unknown error. | Check the injection volume and the Eluent Segment's volume. The total volume used for the run must not exceed the volume of the syringe. If that doesn't solve this error contact the manufacturer for further help. |
| **EVIAL** | "Vial Error" | Vial sensor detected a missing vial at the specified position. | Check the sample rack and make sure, that a vial is placed in the specified position and clear the vial error before continuing (see below). |
| **EVSEQ** | "Vial Sequence Error" | A logical error of the Sample Sequence occurred. | Check the Sample Sequence that all sequence steps in use have a FROM, TO and PROG value >0. Also check that the FROM value is smaller or equal to the TO value. |
| **EDVNO** | "Derivatisation: Vial in wrong rack" | The Vial programmed in the Vial Sequence is in the wrong Rack. | Check the Sample Sequence that they are on the right Rack (Pos. 61-120) only. The left Rack (Pos. 1-60) is used for the mixing vials. |
| **EDRCK** | "Derivatisation: Rack Error" | The Rack sizes entered in the Service Menu are different. | Check the Rack sizes in the Service Menu. Both, Rack 1 and Rack 2 must have the same number of vials in the X- and Y-Axis. |
| **HARDWARE ERRORS** | | | |
| **EVINJ** | "Injection-Valve Error" | An error was detected with the movement of the Injection-Valve. | Contact the manufacturer about replacing the defective part. |
| **EVPRG** | "3-Way-Valve Error" | An error was detected with the movement of the 3-Way-Valve. | Contact the manufacturer about replacing the defective part. |
| **EMOTX** | "X-Axis Motor Error" | An error was detected with the movement of the X-Axis stepping motor. | Contact the manufacturer about replacing the defective part. |
| **EMOTY** | "Y-Axis Motor Error" | An error was detected with the movement of the Y-Axis stepping motor. | Contact the manufacturer about replacing the defective part. |
| **EMOTZ** | "Z-Axis Motor Error" | An error was detected with the movement of the Z-Axis stepping motor. | Contact the manufacturer about replacing the defective part. |
| **EMOTS** | "Syringe Motor Error" | An error was detected with the movement of the syringe stepping motor. | Contact the manufacturer about replacing the defective part. |

# 10. SERVICE & MAINTENANCE

## 10.1 ADJUSTMENT OF NEEDLE POSITION

In case the needle inject position is not found (malfunctioning), check first the tension of the driving belts. If the defect is not caused by them, the needle position has to be adjusted with the help of the SERVICE MENU.

### 10.1.1 MODIFIED SAMPLE RACKS

The autosampler comes with two standard sample racks (if not otherwise ordered) for 120 samples. In case special vials of different shape and dimensions as the standard vials have to be used, they can be ordered separately. The user has to modify only the programming for the sample racks. The sample racks are coded and are placed into the sample chamber without any danger of mixing them up. The positions of the vials are numbered for the standard rack from 1 to 60 and 61 to 120.

The needle has to be programmed via the diagonals between vial no. 1 and 60 as well as between vial no. 61 and 120. For this, the sliding arm has to be moved to these positions (SERVICE PROGRAM #2) and the number of steps needed, to be entered into the SERVICE PROGRAM #1.

WARNING: For this procedure, the autosampler must be connected to the power supply.

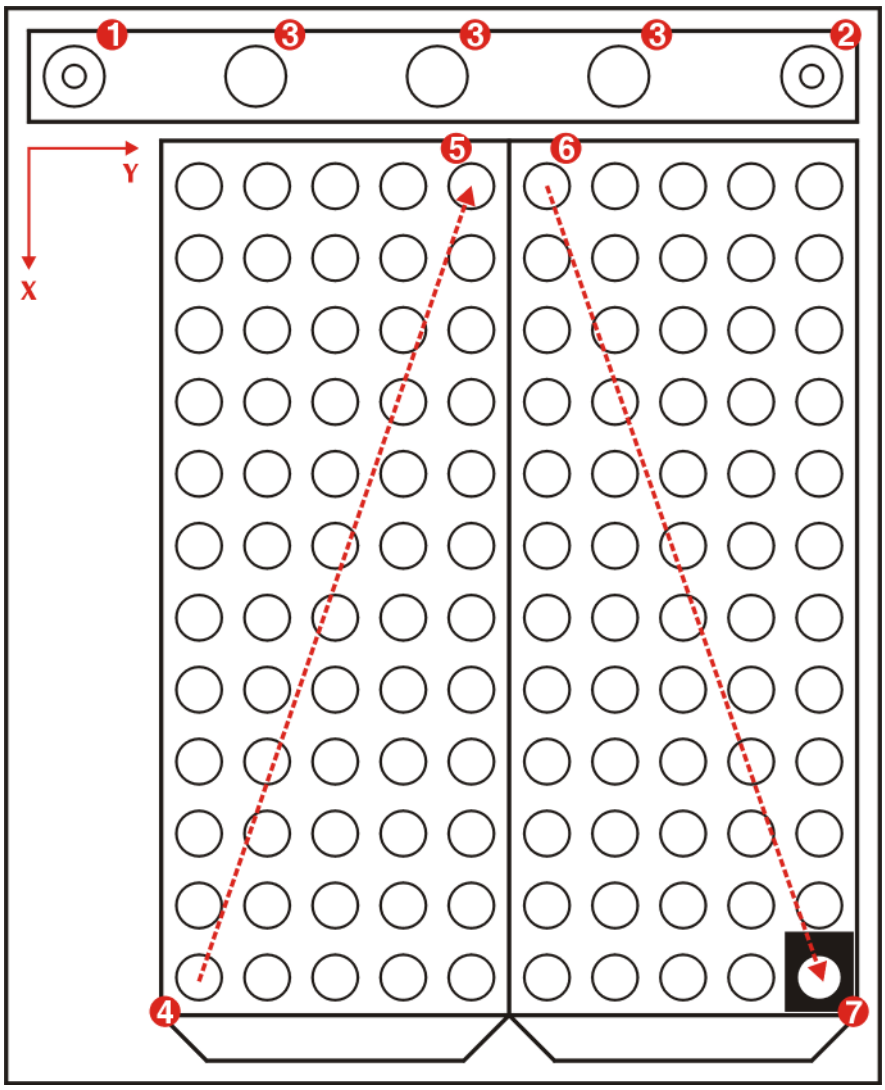
Programming procedure:

- Remove the cover of the autosampler
- Press the Menu key (MAIN MENU)
- Select INSTRUMENT CONFIGURATION (5)
- Press the function key [F2] SERVICE PROGRAM #1
- Enter the legitimation code (‘123’)
- Press key [F3] SERVICE PROGRAM #2
- The sliding arm moves to the home position
- Press key [F4] STEP WIDTH
- Select a reasonable step width (1, 10, 100, 500, 1000) with the [F4] key
- The coordinate is selected with the [CURSOR] keys
- The sliding arm is moved step by step through the [SELECT] keys.
- With the help of these both keys, the sliding arm can be moved along the coordinate requested. For the exact positioning, a step size of 10 and then finally of 1 is recommended.

- The arm has to be positioned in such a way, that the needle injects exactly in the center of vial 1, 60, 61 and 120. The x and y positions of each vial have to be documented and entered in SERVICE PROGRAM #1
- Enter the values in SERVICE PROGRAM #1 for rack no. 1 (x-coordinate of vial 1 at BEGX and y-coordinate of vial 1 at BEGY; x-coordinate of vial 60 at ENDX and y-coordinate at ENDY) and for rack no. 2 (x-coordinate of vial 61 at BEGX and y-coordinate of vial 61 at BEGY; x-coordinate of vial 120 at ENDX and y-coordinate at ENDY)
- The positions for the injection port and purge port are selected as described above and entered in SERVICE MENU #1.
- Also, the SERVICE PROGRAM #1 is used for the setting of the z-axis of the inject position. The z-settings (approx. 15,0 mm) have to be adjusted in very small steps until the needle holder can be pushed downwards (by hand) only approx. 0.5 mm during the injection process. The same z-coordinates have to be entered for the purge position.
- In SERVICE PROGRAM #2 the functions for the inject port and purge port can be displayed and tested by entering the appropriate function numbers.

Note: Don't put your hand into the sample chamber during operation !

In case somebody manipulated in the sample chamber during operation and has accidentally touched the needle - the needle no longer detects the vial positions and inject port - the autosampler has to be switched off immediately and the needle has to be checked for any damage.




**Picture 30: Sample Rack Positions**

1 Purge Port
2 Injection Port
3 Derivatisation Ports
4 Beginning of Rack 1
5 End of Rack 1
6 Beginning of Rack 2
7 End of Rack 2

# 10.2 REPLACING THE INJECTION SYRINGE

The standard version of the Autosampler is using a 500 µl syringe for sample dosing. The piston of the syringe is linearly motor driven which ensures the accurate dosing process. If very small/large volumes should be analyzed, the microliter syringe and the transfer capillary can be replaced.

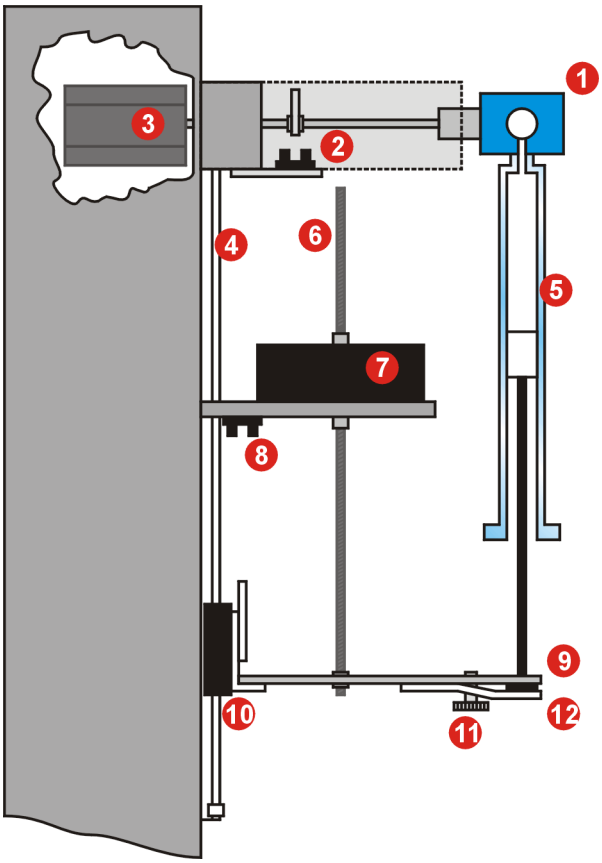
The top of the syringe is screwed into a 3/2-way switching valve. With this valve, the syringe can be connected either to the sample needle or to the wash solution. The piston of the syringe is fixed to a forked support which is driven up and down.

To remove the syringe, unscrew the knurled fitting (11) which fixes the plate-spring holding the piston of the syringe.

Turn very carefully the glass syringe with a suitable spanner until it can be removed.

Fill the new syringe with dest. water, place the end of the piston under the forked support and screw the top end of the syringe into the 3/2-way switching valve. Fix the end of the piston with the knurled fitting again.

To remove any air in the syringe, an analysis run should be done, followed by 6 to 7 washing cycles. For checking the reproducibility of the measured values, a multiple analysis run should be performed.

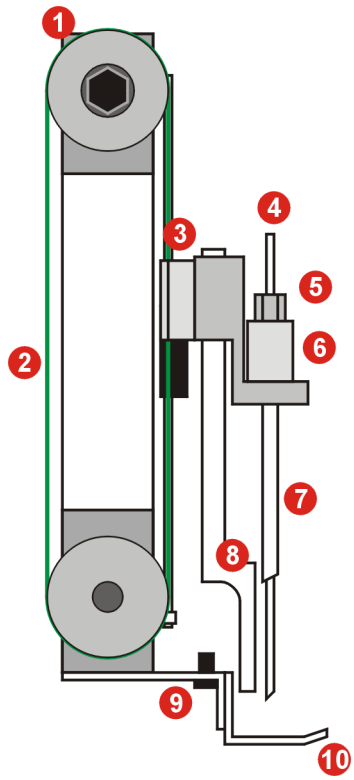



**1** 3-Way Switching Valve (Sample/Wash Solution)
**2** Positioning Sensor (Valve)
**3** Positioning Drive (Valve)
**4** Slide
**5** Syringe
**6** Spindle Driving Shaft
**7** Syringe Motor
**8** Positioning Sensor (Syringe)
**9** Forked Support
**10** Sliding Carriage
**11** Knurled Fitting
**12** Plate Spring

**Picture 31: Syringe Schematic**

# 10.3 REPLACING THE INJECTION NEEDLE

Should the injection needle been damaged or in case the needle gets caught in the top of the sample vial, the autosampler has to be switched off. Check the tip of the needle concerning fine edges etc., if necessary, exchange the needle.




1 Shaft
2 Driving Belt
3 Belt Connection
4 Injection Needle
5 Injection Needle Fitting
6 Air Needle Screw (Top Part)
7 Air Needle (Bottom Part)
8 Vial Sensor Plate
9 Vial Sensor
10 Vial Stripping Stop

**Picture 32: Needle Schematics**

- After finishing the flushing cycle, turn off the autosampler and disconnect the power supply.
- Remove the top cover of the instrument and the covering of the sliding carriage. (The needle is fixed by a tension screw positioned at the angle's lead-in hole. The angle is mounted with 4 screws to the sliding carriage, moving up and down through a driving belt).
- At the upper part of the needle, the fitting with the capillary has to be removed. Use the pliers to unscrew the needle insert. Then the needle can be removed from the top.
- The new sample needle has to be placed into the needle holder. Use the pliers to tighten the needle insert and connect the capillary to the protruding end of the needle.
- The injection needle position has to be checked and adjusted. For this, please refer to the ***10.1 Adjustment of Needle Position***.

**Warning:** For this procedure, the autosampler has to be connected to the power supply. Please adhere strictly to the Service Menu description, otherwise injuries can result if not correctly done.

- Afterwards, the covering of the sliding carriage and the autosampler's cover have to be remounted.
- To clean the needle, start the analyses which should be followed up by 6 - 7 wash cycles. For checking the reproducibility of the system, a succession of analysis runs should be performed.

# 10.4 REPLACING THE SAMPLE LOOP

With variable injection volume, the sample loop is only partially filled with sample. If the fix volume injection mode is selected, the sample volume to be injected is dependent on the size of the sample loop.

For extremely accurate dosage (RSD < 0.5 %) the sample loop should be overfilled with sample. For smaller volumes, the sample loop has to be exchanged:

- Remove the side cover on the right side of the instrument where the syringe and injection valve is positioned.
- Remove the connections of port 1 and 4 of the injection valve. Unscrew the sample loop.
- The new sample loop with the appropriate fittings and ferrules has to be mounted to port 1 and 4.

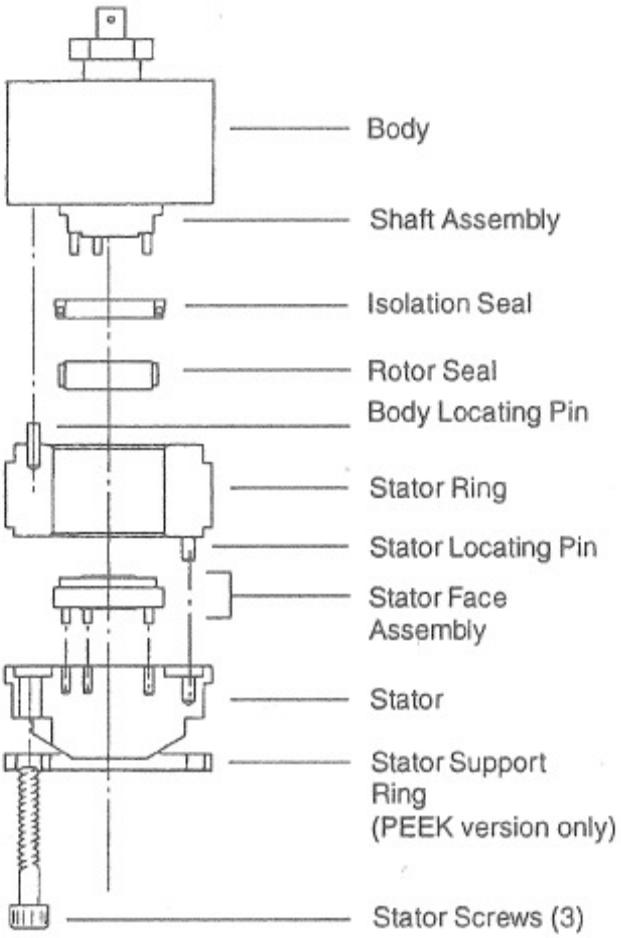
Important: For sample volumes higher than 100 µl, the standard 500 µl syringe should also be exchanged for bigger one.

# 10.5 INJECTION VALVE MAINTENANCE

Abrasion or any lines caused by particles in the fluid can cause leakages at the rotor or stator parts.

## 10.5.1 DISASSEMBLING THE VALVE

Disconnect the Autosampler from the power supply. Loosen all fluid capillaries. Cleanness is of the utmost importance, when disassembling the valve.




**Picture 33: Injection Valve Diagram**

- Unscrew the stator screws.
- Remove the stator assembly by pulling axially to disengage the various parts.
- Remove the rotor seal by prying it off of the four seal pins, using a screwdriver.
- Check if there are any scratches on the rotor's or stator's surface.

## 10.5.2 REASSEMBLING THE VALVE

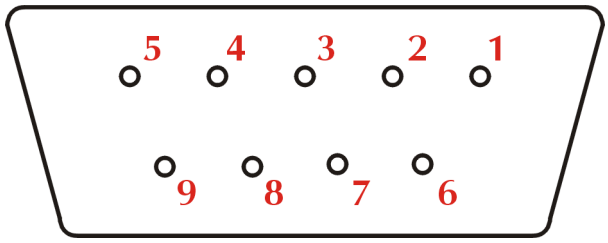
The new rotor seal has to be placed into the position. The rotor seal must be correctly oriented with rotor seal slots facing the stator.

Reassembly the other part and mount the stator. Tighten each of the three screws a little at a time and finally tighten them securely.

Before using the autosampler for analysis runs, the valve must be flushed with the eluent used.

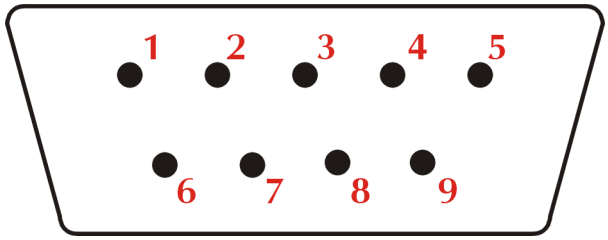
# 10.6 FUNCTION CONNECTORS

## 10.6.1 REMOTE CONTROL



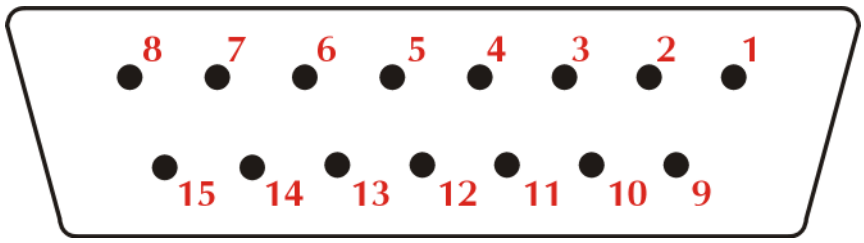

| PIN | FUNCTION |
|---|---|
| 1 | START |
| 2 | STOP |
| 3 | ERROR N.O. |
| 4 | ERROR N.C. |
| 5 | INJECT N.O. |
| 6 | GND |
| 7 | INJECT N.C. |
| 8 | ERROR COM |
| 9 | INJECT COM |

## 10.6.2 RS-232 SERIAL INTERFACE




| PIN | FUNCTION |
|---|---|
| 1 | DCD |
| 2 | RXD |
| 3 | TXD |
| 4 | DTR |
| 5 | GND |
| 6 | DSR |
| 7 | RTS |
| 8 | CTS |
| 9 | RI |

## 10.6.3 AUXILIARY FUNCTIONS




| PIN | FUNCTION |
|---|---|
| 1 | AUX 1 N.O. |
| 2 | AUX 1 COM |
| 3 | AUX 2 N.O. |
| 4 | AUX 3 N.O. |
| 5 | AUX 3 COM |
| 6 | AUX 4 N.O. |
| 7 | AUX 5 N.O. |
| 8 | AUX 5 COM |
| 9 | AUX 1 N.C. |
| 10 | AUX 2 COM |
| 11 | AUX 2 N.C. |
| 12 | AUX 3 N.C. |
| 13 | AUX 4 COM |
| 14 | AUX 4 N.C. |
| 15 | AUX 5 N.C. |

# 11. TROUBLESHOOTING

## 11.1 BAD REPRODUCIBILITY

A bad reproducibility can have different causes like leakage, block-ups in the capillaries, air bubbles in the injection port, defective sealing ring in the inject port or not sufficiently degassed wash solvent.

The following points should be checked:

### WASTE BOTTLE

The waste bottle has to be positioned on the same height as the autosampler.

### DEGASSING OF THE WASH SOLVENT

Even very small air bubbles, especially with small volumes, can affect the reproducibility; Therefore, the wash solvent has to be degassed sufficiently before operation.

### PROGRAMMING THE AIR SEGMENT

The air segment is used for keeping the sample and the eluent segment apart from each other. The volume of the air segment should be exactly selected to ensure the complete separation of the sample and the eluent segment, but should not be injected. With an air segment of 5 µl, a reliable separation of the sample and eluent is secured.

### CAPILLARIES

Check all the fittings (injection valve, capillaries to the injection port, connection to the sample needle, connection to the separation column) for any leakage.

### NEEDLE POSITION

The injection position of the sample needle in the inject port must be exactly in the middle. A not correctly adjusted sample needle can cause premature abrasion of the needle and seal.

In case of a defect needle port seal or block-up of a capillary, the sample volume is not injected completely (leaking of the injection port). A drop remains on the injection position.

The injection needle should also be checked concerning any damage.

### BLOCK-UP OF A CAPILLARY

The transfer of the sample into the needle/transfer capillary can be checked with the help of colored solution and programmable air segments. With a syringe, each capillary can be checked (at the injection valve) and any blockage can be removed.

## BLOCK-UP OF INJECTION NEEDLE

The transfer can be checked as described above. When no liquid shows up in the transfer capillary, the needle may be blocked. This blockages can be easily removed by pushing a small gauge wire through the Injection needle from top to bottom to push out any particles inside the needle.

# 11.2 VIAL ERROR

In case the error message **EVIAL** will be displayed although a vial is placed in this vial position, the needle has to be checked. If necessary, the needle has to be exchanged, as a defective needle can pull out the vial from the rack and the sample arm can be jammed. A bended needle also can cause any damage to the injection port.

# 11.3 NO SAMPLE INJECTION CAN BE DETECTED

Although sample injection is done, no signal can be detected during data acquisition. The function of the injection valve has to be checked in the appropriate service menu and the capillaries checked for any blockage. If necessary the needle depth has to be checked: for standard sample vials approx. 30 mm. The needle is not allowed to touch the bottom of the sample vial!

# 11.4 BENT NEEDLE

The injection needle does not bend under normal operation. When this happens periodically, the reason is most often that the needle does not reach its intended position and come down on some solid object, like the sample trays. The reason for this is dirt/dust on the axis rails; the movement of an axis is noisy and ends at different positions. Clean the rails carefully and put some oil on them. If this doe not solve the problem, contact the manufacturer.

# 12. SPARE PARTS & ACCESSORIES

## 12.1 SPARE PARTS

| Catalog No. | Part |
|---|---|
| 20 21 920 | Dosing Syringe: 500 µl |
| 20 34 003 | 3-Way Valve |
| 20 20 044 | Injection Needle |
| 25 01 137 | Air Needle |
| 24 10 027 | Injection Port Sealing |
| 77 40 006 | Injection Valve (Stainless Steel) |
| 97 40 001 | Injection Valve (PEEK) |
| 77 40 027 | Stator Face Assembly |
| 77 50 016 | Rotor Seal: Injection Valve (Vespel) |
| 77 50 055 | Rotor Seal: Injection Valve (PEEK) |
| 31 01 011 | Stepping Motor: Syringe |
| 23 02 001 | Gear-Belt: Z-Axis |
| 23 02 002 | Gear-Belt: X-Axis |
| 23 02 003 | Gear-Belt: Y-Axis |
| 22 20 006 | Sample Rack 1: 2ml Vials (Pos. 01-60) |
| 22 20 007 | Sample Rack 2: 2ml Vials (Pos. 61-120) |
| 20 60 005 | Front Glass Panel |

## 12.2 SAMPLE LOOPS

### 12.2.1 PEEK

| Catalog No. | Part |
|---|---|
| 21 90 040 | Sample Loop, 5 µl (PEEK) |
| 21 90 041 | Sample Loop, 10 µl (PEEK) |
| 21 90 042 | Sample Loop, 20 µl (PEEK) |
| 21 90 043 | Sample Loop, 50 µl (PEEK) |
| 21 90 044 | Sample Loop, 100 µl (PEEK) |
| 21 90 045 | Sample Loop, 200 µl (PEEK) |

*Other sizes are available on request.*

### 12.2.2 STAINLESS STEEL

| Catalog No. | Part |
|---|---|

| | |
|---|---|
| 77 55 020 | Sample Loop, 5 µl (Stainless Steel) |
| 77 55 021 | Sample Loop, 10 µl (Stainless Steel) |
| 77 55 022 | Sample Loop, 20 µl (Stainless Steel) |
| 77 55 023 | Sample Loop, 50 µl (Stainless Steel) |
| 77 55 024 | Sample Loop, 100 µl (Stainless Steel) |
| 77 55 025 | Sample Loop, 200 µl (Stainless Steel) |

*Other sizes are available on request.*

## 12.3 SAMPLE VIALS

| Catalog No. | Part |
|---|---|
| 70 04 001 | Sample Vial, glass, 1 ml , w. Crimp Cap (Pack: 100 pieces) |
| 70 04 002 | Sample Vial, conical, 200 µl w. Silicon Caps (Pack: 100 pieces) |
| 70 04 006 | Sample Vials, glass, 1.5 ml (Pack: 100 pieces) |
| 70 04 101 | Crimp Caps w. Septum (Pack: 100 pieces) |
| 70 04 102 | Silicon Caps (Pack: 100 pieces) |
| 70 04 103 | Screw Caps w. Septum (Pack: 100 pieces) |