

ÄKTApilot

CHROMATOGRAPHY SYSTEMS

ÄKTApilot™ is a high-performance, automated liquid chromatography system designed for process development, process scale-up, scale-down and small-scale production.

The system has the capacity to purify from milligrams to tens of grams of product and is biocompatible, hygienic and sanitizable.

- Hygienic design
 - Enables purification of microbiologic- and contaminant-free products
 - For convenient Cleaning Validation
- High dynamic capacity
 - Flow capacity 4-400 ml/min with 0-100% gradient
 - 4-800 ml/min flow with limited gradient
 - Possible to purify 10 g product per cycle
- Fast and convenient start to every purification
 - Method wizard for easy programming
 - Preprogrammed cleaning method and column lists
 - Pressure regulation of flow rate at sample application and column packing
- Validation support
 - IQ/OQ documentation available
 - UNICORN™ supports FDA 21 CFR part 11 for Electronic signature and Electronic records
- Wetted parts easily changeable for
 - Convenient product change when campaigning
 - Simplified cleaning validation
- Bench-top design
 - Fits in small areas
 - Easily moved and serviced

The system consists of the ÄKTApilot separation unit, a computer including a flat-screen monitor and UNICORN control system (Fig 1).



Fig 1. ÄKTApilot.

UNICORN ensures quick, simple communication between systems and users and meets the stringent control and data handling procedures of modern production and laboratory facilities. Method wizards provide easy method generation. Optimized methods are transferred easily from laboratory to production scale.

System design and description

The ÄKTApilot components are based on our ÄKTA™ platform of reliability, scalability and flexibility. The UNICORN control system is the most current platform used in our research and production scale systems. ÄKTApilot comes complete with multi-wavelength UV, pH and conductivity detectors, and multiple air and pressure sensors.

The valves and the fittings are specially developed for hygienic, leak-free operation. Electrically actuated diaphragm valves, set together in blocks of four, minimize hold up volumes and prevent dead volumes. An air trap and optional filter ensure protection of the columns.

ÄKTApilot contains all the fluid handling components required to perform eluent delivery and mixing, sample application, and in-line monitoring. All the control and mechanical components are located inside an easily cleaned cabinet.

The flow path consists of two sets of four inlets on the main pump and four inlets on the sample pump. The latter has the same capacity as the system pump, 400 ml/min. The in-line mixer ensures high accuracy of the high-pressure gradient.

The air trap is connected in parallel for easy actuation. It is possible to use two columns in series or one column at a time. Pressure is monitored at four points and it is possible to pressure regulate the flow rate for convenient sample application and column packing. Monitoring of conductivity, UV and pH are all displayed in UNICORN and extra monitors can be connected to the system for special purposes. Fractionation is performed either by the standard setup of eight outlet valves or by adding external valve blocks.

The flow path

1. The sample valve (V3) selects an appropriate sample inlet tubing. Additional valve blocks (EVB) can be added to all inlets. The sample pump (P-908) delivers the sample solution to the column via a rapid responding pressure sensor, the flow
2. The inlet valves (V1+V2) select eluent inlet tubings and external valve blocks (EVB 981) can be added to these inlets. The system pump (P-907) delivers the eluent through a rapid responding pressure sensor (1) and a mixer to the air trap valve (V4). The flow can be directed through or bypass the air trap.
3. After the air trap valve (V4), the eluent flow is directed through an air sensor (1) and a pressure sensor (2) to the flow direction valve (V5). The air sensor is used to prevent air from entering the column. The pressure sensor monitors column head pressure.
4. The flow path continues through one of the column valves (V6+V7) to the column. The flow passes through the column media where the sample purification takes place.
5. The flow then passes via a pressure sensor (4) through the pH electrode located in a pH flow cell, the conductivity cell, and the UV flow cell. The pre and post column pressure sensors continuously monitor the inlet and outlet column pressures and the differential pressure reading on screen.
6. The flow path continues to the outlet valves (V8+V9), which are used to switch the flow to waste or fraction collection. In addition, external valve blocks (EVB 988) can be connected at this position in the flow path.

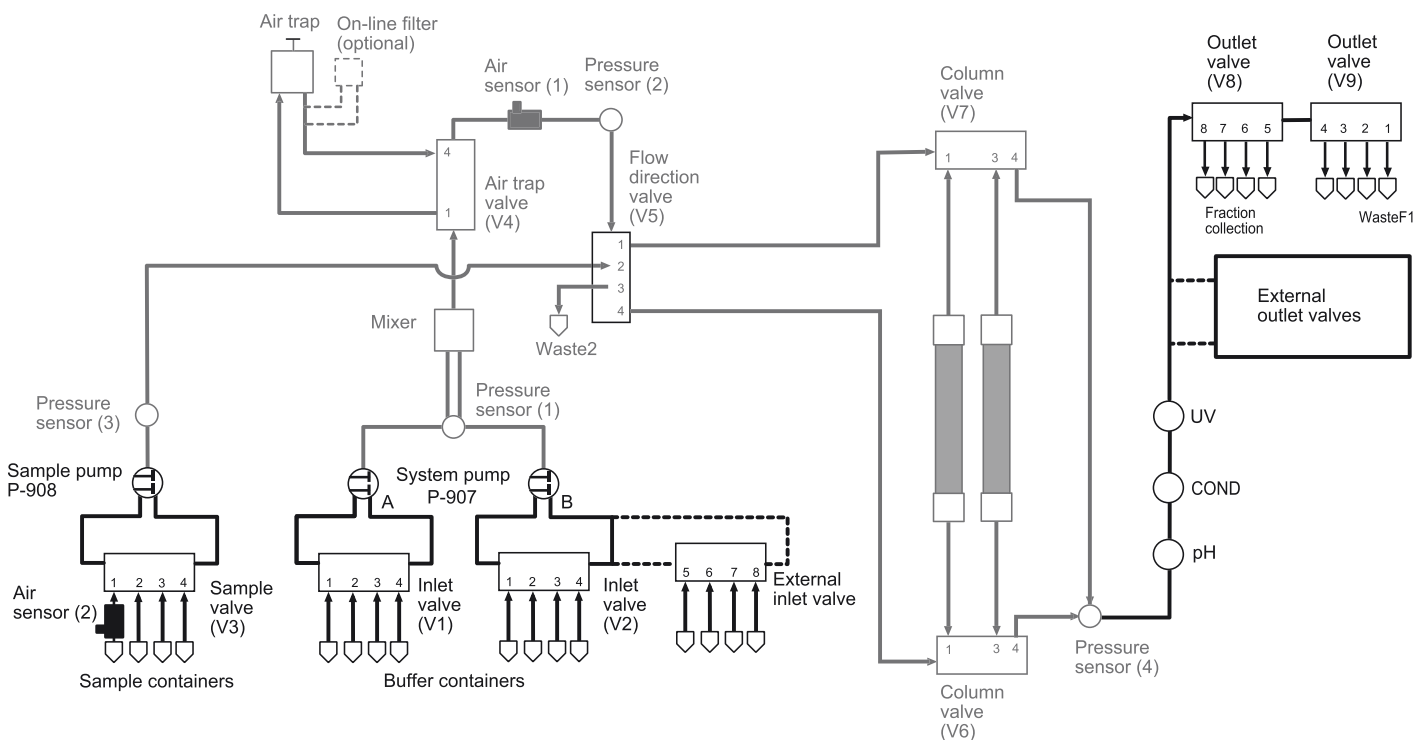


Fig 2. The liquid flow path.

Robust and hygienic design

The ÄKTA platform now offers a system with sanitizable components. All wetted parts can be easily changed to simplify cleaning validation and eliminate cross contamination when the system is used in a multipurpose, campaign-based situation.



Fig 3. Sanitary fittings.

Sanitary tube fittings

The flow through tube fittings are sanitarly designed, with prefabricated molded tube ends and o-ring seals to prevent leakage in or out of the flowpath (Fig 3). The small size of the fittings enables a compact system design.

Valves

Nine blocks of stepper motor actuated membrane valves control the liquid flow in the system. The valves have different numbers of inlet and outlet ports depending on where they are located in the flow path. The valves are built in blocks of four to minimize hold up volumes and dead volumes. Actuator moved diaphragms ensure optimal sanitization (Fig 4).

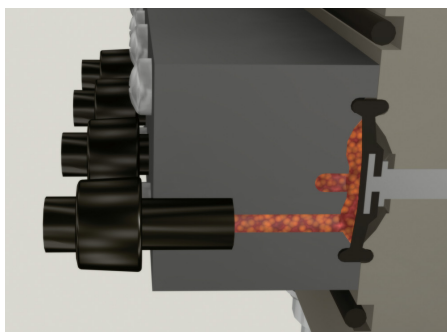
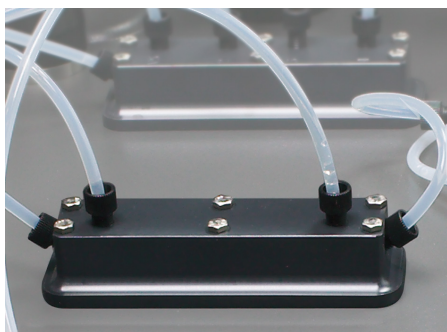


Fig 4. Valves.

External valve blocks

ÄKTA pilot can be modified by adding up to six additional external valve blocks (EVB) on a rack that fits neatly on the side of the instrument (Fig 5). A setup of four additional outlet valve blocks (EVB988) gives 16 outlet valves that allow the collection of 22 peak fractions that can be selected in the wizard. Two inlet valve blocks (EVB981) gives eight additional inlet valves to allow maximum use of system pumps to a capacity of 800 ml/min. In addition, increased inlet capacity also allows twice as many buffers or CIP solutions to be used.



Fig 5. External valve blocks.

The system and sample pumps

The high-performance system pump P-907, accurately controls liquid flow. The pumpheads are sanitarly designed with a self-contained rinsing system to prevent contamination and pump damage. Low shearing forces ensure that cellular material is not damaged during operation. The system is equipped with two 400 ml/min pump modules that produce accurate gradients with minimum gradient lag volume. The two pumps allow for 800 ml/min isocratic and limited gradients when used together.

All three system pumps have high resistance seals that withstand 20 bar and are solvent resistant. Excess pump pressure is monitored by a rapid response pressure sensor and is relayed to a preset alarm function in UNICORN. The sample pump, P-908, is identical to the system pump but only has one pump module (Fig 6). It is also equipped with a pressure sensor.

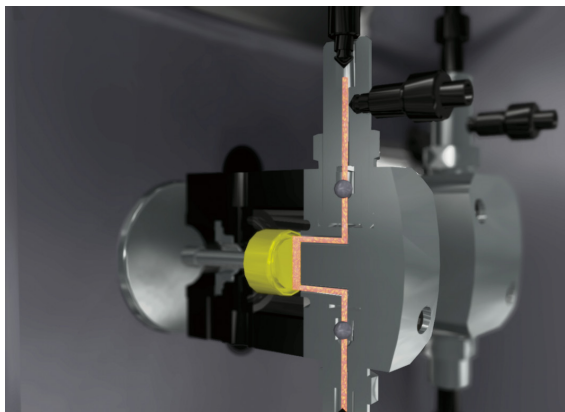


Fig 6. Pump.

pH, conductivity and UV sensors

ÄKTApilot is equipped with sanitarly designed sensors for continuous in-line measurement of pH, conductivity and UV absorbance. The detectors provide accurate and reliable monitoring through self-test and self-calibration. The flow cells are placed close together, which minimizes band broadening and time delay between the detectors. The flow cells are easily accessible from the front panel to facilitate maintenance.

The UV cell can measure up to three wavelengths simultaneously, in the range 190–700 nm, via fiber optics (Fig 7). The pH electrode is mounted in the pH flow cell after pressure sensor 4 in the flow path. The pH monitor measures in the range 2–12. The conductivity cell is placed after the pH electrode in the flow path. It is used to verify gradients, follow peak positions relative to ionic strength and measure temperature via a built-in sensor.

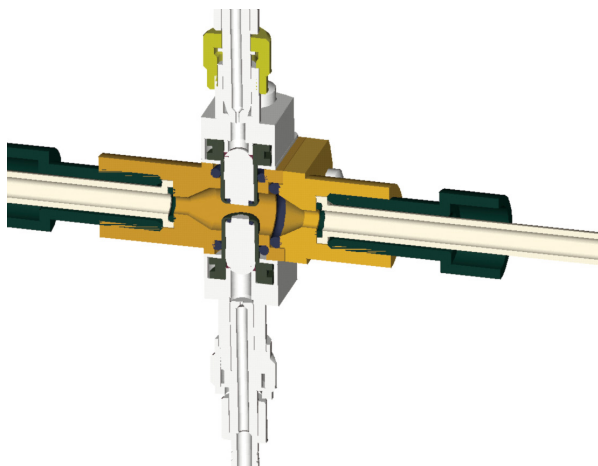


Fig 7. UV cell.

Pressure and air sensors

The system has four pressure sensors for measurement of the pressure. Two of the sensors are placed just before and after the column to read the pressure differential across the column. The two remaining pressure sensors are placed after the pump to secure fast stop of flow at high pressure.

There are two different air sensors in the system, one for sample application and one for column protection. The air sensors are based on ultrasonic technology with clean flow through properties and have the same functionality as those found in ÄKTA and BioProcess™ systems.

Fast and convenient start to every purification

The UNICORN system control with Evaluation and Reporting, secures a simple user interface and a high level of automation. The Method Wizard, Multifile Peak Compare Wizard and preprogrammed cleaning methods provide a high degree of efficiency in scale-up and production work (Fig 7). The software has networking capabilities to put you in total control from laboratory-scale research to full-scale production. UNICORN offers functions that are vital for both R&D and production.

UNICORN Control System has undergone an independent audit by Weinberg Associates and is certified for use in a FDA 21 CFR Part 11 and GMP compliant environment. The Electronic Signature and Record System utilizes a dual password confirmation, documenting locking scheme and traceable audit trail.

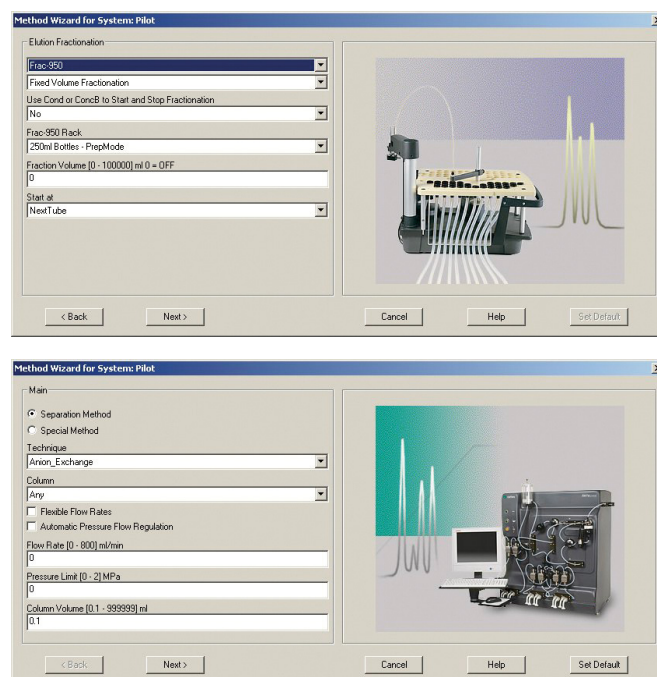


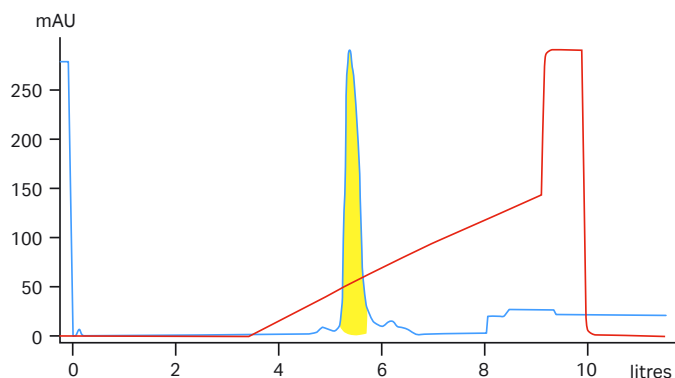
Fig 8. UNICORN Method Wizards.

The Method Wizard, together with a flexible system configuration, allows fast and easy purification or column packing. The system supports you in process development, scale-up/-down and pharmaceutical production. The Scouting feature gives automatic support in process development and optimization.

Scaling up to production with ÄKTApilot and optimized methods from UNICORN

UNICORN cuts preparation times to a minimum whilst maintaining accuracy and assuring reproducibility. This allows quick and simple transfer from one stage of a project to the next. For example, purification of Protein Tyrosine Phosphatase 1B (PTP 1B) expressed in *Escherichia coli* uses a protocol that was developed and optimized with UNICORN on ÄKTApilot 100 chromatography system. The same purification process with three steps (capture, intermediate purification and polishing) was then scaled up for use on ÄKTApilot.

System: ÄKTApilot
Column: FineLINE™ 70
Media: SOURCE 30 Q
CV: 0.4 l
Sample volume: 1.7 l, 4.25 CV
Liquid velocity: 140 cm/h
Buffer A: 25 mM Tris-HCl, 2 mM EDTA, pH 7.5
Buffer B: 25 mM Tris-HCl, 2 mM EDTA, 1.0 M NaCl, pH 7.5



System: ÄKTApilot 100
Column: XK 16/20
Media: SOURCE 30 Q
CV: 20 mL
Sample volume: 27 mL, 1.35 CV
Liquid velocity: 140 cm/h
Buffer A: 25 mM Tris-HCl, 2 mM EDTA, pH 7.5
Buffer B: 25 mM Tris-HCl, 2 mM EDTA, 1.0 M NaCl, pH 7.5

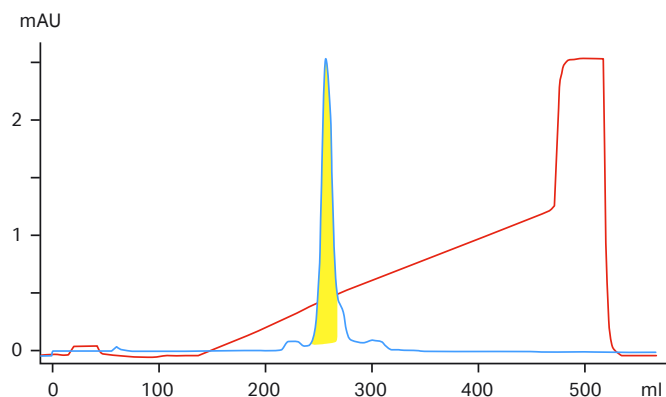


Fig 9. Chromatograms from ÄKTApilot and ÄKTApilot 100 of the polishing step using SOURCE 30Q.

During the scale-up, loading (mg protein/ml media) and linear flow rate were kept constant. All chromatography steps were run in packed bed mode. The media used for the capture step was Q Sepharose™ XL. Intermediate purification was performed by hydrophobic interaction chromatography with Phenyl Sepharose High Performance. The collected fractions were desalted with Sephadex™ G-25 F before the polishing step with SOURCE™ 30Q. A total amount of 0.132 g and 3.5 g PTP 1B was recovered on the ÄKTApilot 100 and the ÄKTApilot systems, respectively (Fig 9).

Trouble free sanitization

The efficiency of the sanitization of ÄKTApilot was tested by subjecting a sanitization program, which uses 1 M sodium hydroxide (NaOH) as an antimicrobial agent, to microbial challenge tests. The system was infected with solutions containing three strains of bacteria that are recommended by the United States Pharmacopoeia (USP 25) and a strain of yeast commonly used in production environments (Table 1). The USP 25 requires a log 6 reduction of colony forming units.



Fig 10. Microbial challenging test.

Table 1. The challenging organisms

<i>Escherichia coli</i>	ATCC 8739 Gram negative bacteria
<i>Pseudomonas aeruginosa</i>	ATCC 9027 Gram negative bacteria
<i>Staphylococcus aureus</i>	ATCC 6538 Gram positive bacteria
<i>Pichia pastoris</i> ¹	GS 115 Yeast

¹ The yeast, *Pichia pastoris*, was selected in favor of the USP 25 recommended yeast *Candida albicans* as it is more frequently encountered in process and production conditions.

Sanitization was performed by circulating 1 M NaOH in the system for approximately 2 h. Then efficiency of the method was evaluated by microbial sampling at predetermined test sites.

The sanitization method showed good efficiency and fulfilled the USP 25 requirements.

Validation documentation & services

Regulatory authorities expect companies that produce therapeutic or diagnostic products to qualify equipment before use. Fast Trak Validation™ services can supply validation documentation to help simplify and speed these procedures.

Fast Trak also supplies installation and operational qualification (IQ/OQ) packages for formal qualification required by current Good Manufacturing Practices (cGMP) and inspection by regulatory authorities. Fast Trak IQ/OQ packages consist of professionally developed templates that will facilitate equipment qualification. The templates have help texts and are easy to complete.

ÄKTApilot – System specifications

System flow rate	
Isocratic and gradient flow double mode	4–400 ml/min Up to 800 ml/min
Operating pressure	Up to 2 MPa (20 bar, 290 psi) between system pump and column inlet
UV wavelength range	190–700 nm in steps of 1 nm, 206–700 nm with ÄKTApilot cell, 3 wavelengths simultaneously
pH range	0–14 (spec. valid between 2–12)
Conductivity range	1 µS/cm to 999.9 mS/cm
Holdup volume	Up to 31 ml
Max backpressure	< 0.06 MPa before column /400 ml/min < 0.045 MPa after column /400 ml/min
Solvent compatibility	All commonly used chromatographic solvents
Degree of protection	IP 24
Size	750 mm × 540 mm × 900 mm (W × D × H)
Weight	114 kg (arm not included)

ÄKTApilot – Technical specifications

System pump and sample pump specifications

Flow rate	
Isocratic and gradient mode	4–400 ml/min
Isocratic and limited gradient	Up to 800 ml/min
Pressure	0–2 MPa (20 bar, 290 psi)
Flow rate accuracy	< ±2% (4–400 ml/min, 0.1–2 MPa)
Flow rate reproducibility	rsd < 0.5% (4–400 ml/min, 0.1–2 MPa)
Viscosity	Max. 5 cP
Gradient composition accuracy	±3% at 8–400 ml/min
Hold up volume	615–1760 µl

UV flow cell specifications

Path length	1 mm, 2 mm, 5 mm
Max. pressure	2 MPa (20 bar, 290 psi)
Max. backpressure	0.03 MPa (0.3 bar, 4.2 psi)
Wavelength accuracy	±2 nm
Wavelength precision	±0.01 nm
Hold up volume	300 µl

pH flow cell specifications

pH range	0–14 (spec. valid between 2 and 12)
Response time	Max. 10 s (0–95% of step)
Max. pressure	0.5 MPa (5 bar, 72 psi)
Max. backpressure	0.01 MPa (0.1 bar, 1.4 psi)
Hold up volume	240 µl

Membrane valve specifications

Max. pressure	2.2 MPa (22 bar, 319 psi)
Max. backpressure	0.015 MPa (0.15 bar, 2 psi)
pH stability range	0–14 (spec. valid between 2–12)
Valve principle	Stepper motor-controlled membranes
Hold up volume	145–354 µl

Conductivity cell specifications

Conductivity range	1 µS/cm to 999.9 mS/cm
Deviation from theoretical conductivity	Max. ±2% of full scale range or ±10 µS/cm whichever is greater in the range 1 µS/cm to 300 mS/cm
Response time	Max. 3 s (0–95% of step)
Temperature sensor	
Accuracy	±2.0°C
Drift	±0.5°C per 10 h
Max. backpressure	0.02 MPa (0.2 bar, 2.9 psi)
Hold up volume	180 µl

Mixer specifications

Max. backpressure	0.05 MPa (0.5 bar, 7 psi)
Mixing principle	Chamber with a stirrer
Hold up volume	4.2 ml

Pressure sensor specifications

Max. pressure	2.5 MPa (25 bar, 363 psi)
Max. backpressure	0.06 MPa (0.6 bar, 9 psi)
Hold up volume	331 µl 2-port 346 µl 3-port

Ordering information

Product	Code No.
ÅKTApilot	29-0086-12*
Additional items	
EVB 981 (Inlet)	28-4079-75
EVB 988 (Outlet)	28-4079-78
EVB Rack	28-4079-72
CIP Manifold	28-4009-03
ÅKTApilot Tubing Kit Column	18-1167-68
Cond. Cell I.D.2.5 mm ÅKTApilot	18-1169-00
ÅKTApilot Valve Membrane CPL	18-1169-10
ÅKTApilot Elbow 90 TC25 Short	18-1169-19
ÅKTApilot TUBE S7 CPL	18-1169-71
ÅKTApilot TUBE S8 CPL	18-1169-75
Inline filter house	18-1171-06
Wetted parts kit	18-1171-07
pH electrode	18-1168-77
O-ring, top air trap	18-1169-12
Connector M6 fem. – 5/16 fem.	18-1169-17
Connector M6 fem. – 5/16 male	18-1169-16
Clamp TC 25	18-1169-18
Connector TC – 5/16 fem.	18-1169-22
Connector TC – 5/16 male	18-1169-23
TC-gasket 25/4 mm	18-1169-24
TC-gasket 25/6.5 mm	18-1169-25
T-connector 5/16 – 24	18-1170-59
Connector 5/16 fem. – 5/16 fem.	18-1173-51

* Preferred UNICORN version should be ordered on separate code number

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