

ÄKTApurifier

ÄKTApurifier™ core systems (Fig 1) are versatile, modular liquid chromatography systems for fast and reliable separations of proteins, peptides, and nucleic acids by FPLC™. The four available core systems can be combined with ÄKTAdesign™ automation kits and hardware to achieve tailor-made solutions for your protein purification needs. Using ÄKTAdesign automation kits to expand ÄKTApurifier core systems also eliminates a number of common problems in FPLC purification, such as long sample loading time, frequent column cleaning, and air corrupting the system.

- Four core system configurations for greater versatility
- High-performance purification by FPLC
- System configurations designed to meet individual research needs
- ÄKTAdesign automation kits can be added to provide greater flexibility
- Greater flexibility keeps costs down – you only buy what you need

Wide range of purification scales

ÄKTApurifier 10 and UPC 10 configurations are designed for purification of microgram to milligram quantities of protein, operating at flow rates of 0.001 to 10 ml/min at pressures of 0 to 25 MPa. ÄKTApurifier 100 and ÄKTApurifier UPC 100 systems can purify gram quantities of protein, running at flow rates of 0.01 to 100 ml/min and pressures of 0 to 10 MPa.

UNICORN control

ÄKTApurifier systems are part of ÄKTAdesign, a range of liquid chromatography systems for academic and industrial research laboratories. ÄKTAdesign systems are controlled by UNICORN™, a control, evaluation, and reporting platform.

UNICORN ensures quick, simple communication between systems and users and meets the stringent control and data handling procedures of modern laboratories. Optimized methods are transferred easily to production scale.



Fig 1. ÄKTApurifier UPC (above) and ÄKTApurifier (below)



Easy access in the lab

ÄKTApurifier systems require minimal bench space, with each system mounted on a swivel platform for easy access to every side. Columns are snapped in place on the outside of the system, either individually or as groups held in column holders.

System configurations provide flexibility

Figure 2 shows a possible configuration of ÄKTApurifier. The setup includes the following:

- Buffer selection kit
- Sample application Kit
- Column screening Kit
- Reverse flow Kit
- Fractionation Kit
- Fraction Collector Frac-950

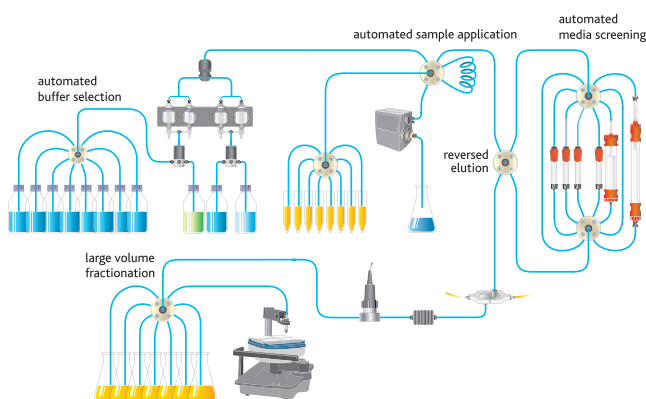


Fig 2. Possible configuration for ÄKTApurifier.

Standard system components

Pumps provide reliable results

The main challenges of a chromatography system pump are to achieve accurate gradient formation over a broad flow rate range and avoid introducing air into the system. ÄKTApurifier 100 and UPC 100 systems use Pump P-901, which operates at high flow rates and at pressures up to 10 bar. ÄKTApurifier 10 and UPC 10 systems use Pump P-903, which operates at the lower flow rates and higher pressure required for running high-resolution analyses at laboratory scale.

Both pumps enable accurate, reproducible flow and gradient formation over a wide flow rate range (Fig 3). The pumps can be used for loading samples larger than 50 ml, but for easy, routine handling of large sample volumes, Sample Pump P-960 is recommended.

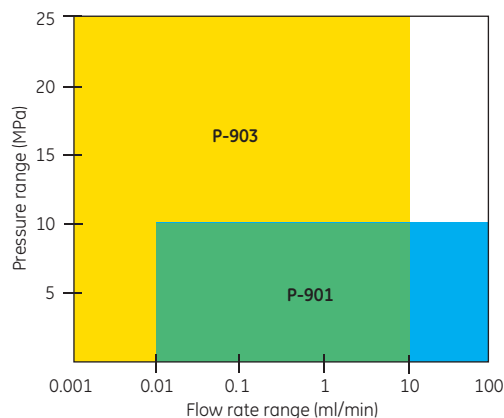


Fig 3. Flow rates and pressure ranges of P-900 series pumps.

Versatile monitoring provides fast peak identification

ÄKTApurifier 10 and 100 core systems include Monitor UV-900 for multi-wavelength UV-Vis detection. User-selected multi-wavelength monitoring enables inline detection of specifically labeled products (Fig 4) and can be used to detect contaminants at higher sensitivity wavelengths (e.g. 280/214/254 nm).

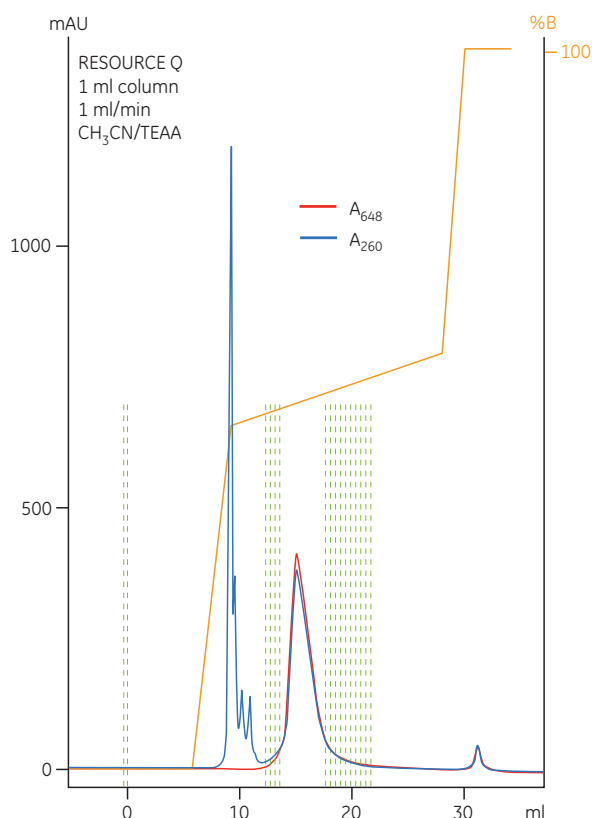


Fig 4. Purification and online detection of Cy5TM labeled (CyDyeTM) 20-mer oligonucleotide.

ÄKTApurifier UPC 10 and 100 core systems include Monitor UPC-900, which combines monitoring of UV absorption, pH, and conductivity. The monitor features fixed wavelength of 254, 280 nm (Hg lamp) or 214 nm (Zn lamp). Other wavelengths can be measured by changing the filter. Two alternative flow cells with path lengths of 2 mm and 5 mm are available.

Improved system functionality with ÄKTAdesign automation kits

All four ÄKTApurifier core systems can be expanded using an extensive range of ÄKTAdesign automation kits and hardware, which enable customization of systems to suit different purification needs.

New functions to enhance system capabilities or create new system configurations are easily added. Functions such as hardware components and software instructions are selected, installed, and implemented by the user. UNICORN controls and monitors the new functions in the same way as all other ÄKTAdesign components. Modification of the system using ÄKTAdesign automation kits greatly improves automation and achieves:

- automatic sample application
- automatic column selection and screening
- automatic buffer and reagent selection
- easy column and media screening
- reversed flow purification in affinity chromatography and protein concentration
- increased security for programmed events – using air sensors

Sample application Kit

Sample application Kit allows for automated sample application of small and medium sample volumes, as well as direct load of large sample volumes. Sample application is easily automated by adding the valve and sample pump included in the kit. Depending on the sample volume, several setups are possible. The flow rate range allows rapid sample application directly to the column or via a Superloop™ or a capillary loop.

Buffer selection Kit

Buffer selection Kit offers automated buffer/reagent screening for purification optimization. Buffers for cleaning can be added and stored in place. Changing buffers manually and moving inlet tubing is no longer necessary

and the risk of introducing air into the system is reduced. A solution of nickel sulfate applied via the buffer valve enables automated charging of metal ion affinity columns.

BufferPrep Kit

BufferPrep Kit eliminates the time-consuming manual buffer preparation and titration usually needed for every pH change in chromatography. For any pH and salt concentration entered, BufferPrep automatically calculates and prepares the composition of the buffer on-line, from four stock solutions. Linear and step salt gradients can be run and pH can be used as a variable scouting parameter. New buffer substances can be added to the buffer library and used by BufferPrep to create the required elution pH conditions.

The accuracy of pH is crucial in many applications. Figure 5 shows BufferPrep used on ÄKTApurifier 100 at 60 ml/min to test seven pH conditions in order to find the optimal pH for elution of a pure Fab fragment. BufferPrep Kit allows greatly increased reproducibility and reliability of obtained results by eliminating and correcting errors that occur when producing buffers manually.

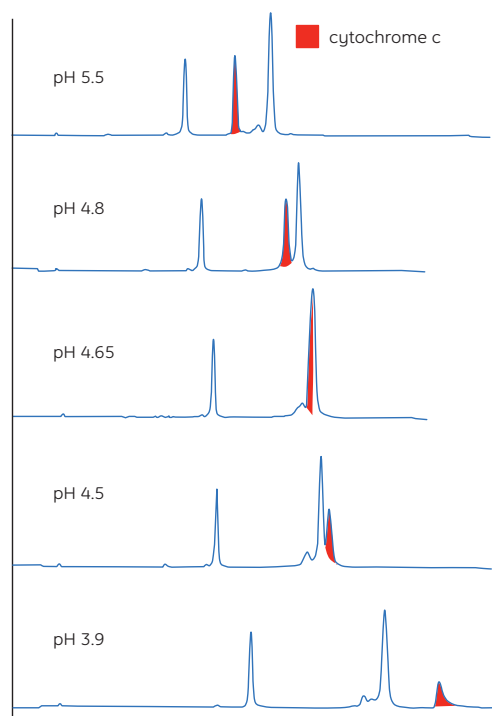


Fig 5. Small changes in pH significantly change chromatographic results. Above, a change of 0.15 pH units alters an elution profile.

BufferPrep can be used for pH scouting in rapid method optimization. The UNICORN method Wizard makes programming of automatic eluent pH scouting for ion exchange chromatography easy and reliable.

Air sensor Kit

The Air Sensor Kit enables safe exclusion of air from the ÄKTApurifier system during the purification. The components of Air sensor Kit are controlled by UNICORN software, which detects air in the system, and pauses the purification run. The kit therefore prevents column damage and ensures that the sample is completely injected.

In addition, UNICORN is programmed to ensure that a chromatographic elution begins only when the completion of sample application has been detected by an air sensor. Variable sample volumes can be applied with no risk of air entering the system or column.

Column screening Kit

Column screening Kit adds two motorized valves, allowing automated column scouting/media screening. Up to eight columns can be connected and column performance can easily be compared while changing conditions in a run. The set-up saves time and increases reproducibility.

Fractionation Kit

Fractionation Kit allows for flexible fractionation of large volumes in the same setup. Using the fractionation valve contained in the kit, up to eight vessels of any size can be used to collect fractions over the whole flow range of ÄKTA design systems.

Reverse flow Kit

In affinity-based protein purification, step elution is preferentially carried out in the reverse direction to sample application. Reverse flow Kit provides an additional motor valve to enable reversed flow through a column. This allows strongly bound substances to elute more effectively and at higher concentration. Reversed flow can also rinse away particles on top of the column, leading to improved column performance. Expanded bed adsorption applications also become possible with reversed flow.

Build on ÄKTApurifier core systems with optional hardware

Monitor pH/C-900

Optional for ÄKTApurifier 10 and 100 core systems is Monitor pH/C-900, a combined monitor for accurate, inline measurement of pH, conductivity, and temperature. The monitor records real-time information about chromatographic conditions. Monitor pH/C-900 provides an accurate response, coupled with high precision over a wide measuring range. This makes Monitor pH/C-900 an excellent choice for use in all chromatography techniques, from reversed phase chromatography with very low conductivity eluents to hydrophobic interaction chromatography in high salt solutions.

Monitor pH/C-900 consists of a control unit, an optional flow cell for conductivity and temperature, an optional flow cell with a holder for the pH electrode, and the pH electrode.

Fraction collectors

Frac-950 (Fig 6) prevents sample loss across the entire flow rate range of ÄKTA design systems, from 0.001 ml/min to 100 ml/min, and the highest flexibility in the choice of collection mode. Fractions are collected in microtitre plates, a variety of test tube sizes, in 20 large vessels (up to 250 ml per fraction) or even funnels for unlimited fraction size. Collection is in volume or time mode and different fraction sizes can be collected during different stages of a separation. Automatic peak fractionation, based on peak detection using level or slope sensing, minimizes peak dilution and cross-contamination.



Fig 6. Fraction collector Frac-950 allows of fractions in a wide range of tubes and 96-well microplates.

Autosamplers

Autosamplers give you more time to work on other projects during the day, and increase throughput by enabling overnight purification runs. Depending on your needs for sample injection, Autosampler A-900 and A-905 for ÄKTAdesign systems offer a variety of alternatives. These autosamplers offer different balances between reproducibility and accuracy and allow flexible use of vials and microplates. Almost every type of vial with an o.d. of 12 mm or 7 mm can be used on Autosampler A-900. Autosampler A-905 handles standard and deep-well 96-well microplates, as well as 384 microvials. Sample loss is kept to a minimum or eliminated by the novel method of sample withdrawal. The Peltier cooling option is available for A-905 and effectively protects heat-sensitive biomolecules during sampling.

Autosampler A-900 and Autosampler A-905 are directly controlled by UNICORN software.

AD-900 Analog/Digital Converter

AD-900 Converter is an analog/digital converter for connecting external instruments to UNICORN control software. The AD-900 Converter allows easier monitoring of signals received as voltage inputs from external sources such as fluorescence detectors, radioactivity monitors, refractive index monitors, or diode arrays.

Reliable system control with UNICORN

Fast and easy start to purification

ÄKTApurifier core systems are entirely controlled by UNICORN software, enabling a fast and easy start to FPLC purification. Users are able to create complete purification schemes for a range of biomolecules by using the method Wizard. This makes ÄKTApurifier extremely easy to use and speeds up routine purifications. It reduces the need for expertise in programming, in chromatography, and in time-consuming literature searches, as all this knowledge is built into the system.

The UNICORN method Wizard in ÄKTApurifier provides a unique combination of flow configuration and methods support for all chromatographic techniques including gel filtration, anion and cation exchange, hydrophobic interaction, affinity, and reversed phase chromatography. Using input parameters such as components in use, flow rate, sample volume, or gradient, the method Wizard builds up the method to suit your needs (Fig 7).

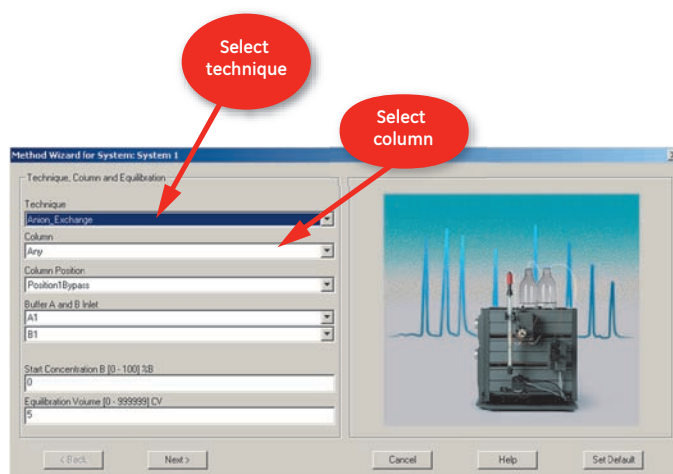


Fig 7. A new method is created in the UNICORN method Wizard in less than 3 min.

The method Wizard eliminates time-consuming and repetitive method programming and uses column volume as a programming base for easy use with any size column.

Routine runs quickly optimized

To quickly establish routine runs, the method Wizard supports programming of method scouting (Fig 8). These scouting methods make it easy to systematically vary run parameters in repeated, automated procedures, reducing optimization times.

UNICORN networks

UNICORN control system has full networking capability, with high security and maximum compliance with regulatory needs. Network support allows control of any ÄKTAdesign system from any connected UNICORN workstation. System control and data access via a PC network is extremely easy to use and gives a complete overview of operations.

With defined access rights, operators connect to and control systems from any remote control or local PC. The user interface is exactly the same. Results can be automatically saved on a server and evaluation and generation of reports can be made locally or at remote PCs.

ÄKTAdesign service

Regular, planned maintenance increases productivity by keeping a system in top working condition and minimizing downtime. A service agreement lets users budget for all service and maintenance costs, giving full cost control and eliminating unexpected expenses. Service agreements greatly simplify compliance with regulatory and quality demands.

Scheduled preventive maintenance carried out by qualified engineers and the built-in service diagnostics of ÄKTAdesign systems provides users with support to meet the requirements of Good Laboratory Practice (GLP).

ÄKTAdesign systems are supported with a comprehensive range of after-sales service agreements that can be customized to individual needs. All ÄKTAdesign instruments are supported in the laboratory by our equipment specialists to minimize operational disruption and ensure functionality of ÄKTAdesign equipment.

Application—pH scouting and two-step purification using ÄKTApurifier 10

ÄKTApurifier combined with BufferPrep Kit was used for anion exchange chromatography to optimize pH for purification of fish plasma vitellogenin (Vtg) from three estradiol-treated teleost fish: rainbow trout, gudgeon, and chub. Optimization of pH conditions was performed using HiTrap Q FF, 1 ml; pH 8.5 provided best resolution and protein binding (Fig 12).

After pH optimization, RESOURCE™ Q was used for capture and intermediate purification (Fig 13). Gel filtration using Tricorn™ Superdex™ 200 was used in the final polishing step to further separate the degradation product from the native form of the Vtg and to store the final product in the desired buffer (Fig 14).

Sample: 250 µl of clarified estradiol (E2)-treated vitellogenin (Vtg) fish plasma sample
Start buffer: 100 mM Tris-HCl, 1 mM PMSF, pH 7.0–8.5
Elution buffer: 100 mM Tris-HCl, 1 mM PMSF, 50 mM NaCl, pH 7.0–8.5
Flow rate: 5 ml/min
System: ÄKTApurifier 10

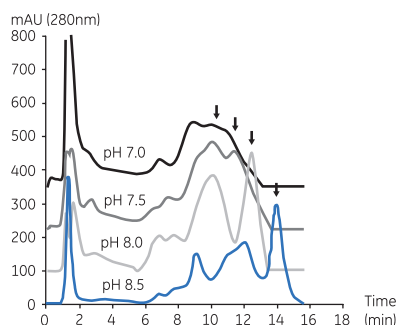


Fig 12. Screening for optimal pH for purifying fish plasma vitellogenin using ÄKTApurifier 10. Optimal pH for the purification was found to be pH 8.5.

Sample: 10 ml of clarified E2-treated fish plasma vitellogenin
Column: RESOURCE Q
Start buffer: 100 mM Tris-HCl, pH 7.0–8.5
Elution buffer: 100 mM Tris-HCl, 500 mM NaCl, pH 8.5
Flow rate: 4 ml/min
System: ÄKTApurifier 10

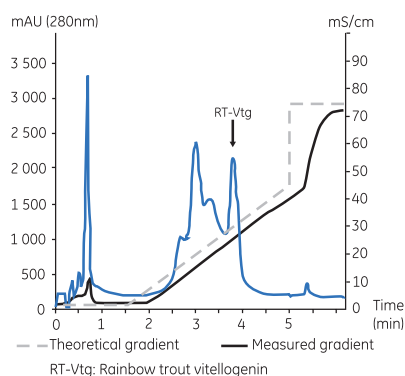


Fig 13. Capture/intermediate purification of vitellogenin on ÄKTApurifier 10 by anion exchange chromatography.

Sample: Vtg fractions of pooled sample from RESOURCE Q capture/intermediate purification step (see Fig 14)
Column: Tricorn Superdex 200 10/300 GL
Elution buffer: 0.05 M carbonate-bi-carbonate, pH 9.6
Flow rate: 0.2 ml/min
System: ÄKTApurifier 10

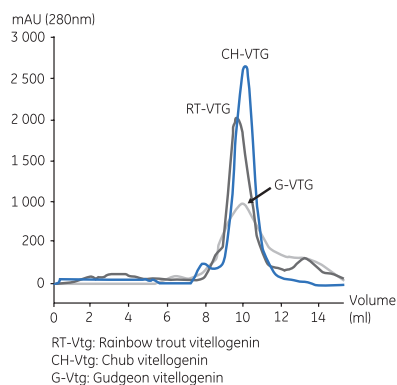


Fig 14. Final polishing of vitellogenin by gel filtration using ÄKTApurifier 10.

System specifications

ÄKTApurifier

Flow rate range setting, ÄKTApurifier 10	0.001 to 10 ml/min
Pressure range, ÄKTApurifier 10	0 to 25 MPa
Flow rate range setting, ÄKTApurifier 100	0.01 to 100 ml/min
Pressure range, ÄKTApurifier 100	0 to 10 MPa
Conductivity range (RPC – IEX – HIC gradients)	1 µS/cm to 999.9 mS/cm
pH range	2 to 12
Temperature range	4°C to 40°C
Solvent compatibility	all commonly used chromatographic solvents
Size (W × H × D)	500 × 620 × 460 mm
Weight	75 kg

Operating data

Pump P-903

Flow rate range	0.001 to 10 ml/min
Increment	0.001 ml/min
Pressure range	0 to 25 MPa (250 bar, 3625 psi)
Pressure limits	programmable upper and lower limit
Internal volume	< 600 µl/pump module
Viscosity	maximum 5 cP

Pump P-901

Flow rate range	0.01 to 100 ml/min
Increment	0.01 ml/min
Pressure range	0 to 10 MPa (100 bar, 1450 psi)
Pressure limits	programmable upper and lower limit
Internal volume	< 1800 µl/pump module
Viscosity	maximum 5 cP

Monitor UV-900

Wavelength range	190 to 700 nm in steps of 1 nm, 3 wavelengths simultaneously
Bandwidth	4 nm
Wavelength accuracy	± 2 nm
Wavelength reproducibility	± 0.01 nm
Linearity	< 2% deviation up to 2 AU at 260 nm with Uracil at pH 2
Noise* (at 230 nm)	< 6 × 10 ⁻⁵ AU
Drift (at 254 nm)	< 2 × 10 ⁻⁴ AU/h
Max. pressure	2 MPa (20 bar, 290 psi)

Monitor pH/C-900

<i>Conductivity unit</i>	
Conductivity range	1 µS/cm–999.9 mS/cm
Noise	± 0.5% of full-scale calibrated range
<i>pH unit</i>	
pH range	0 to 14 (specifications valid between 2 and 12)
Accuracy	± 0.1 pH unit, temperature compensated
Stability	max 0.1 pH units deviation/10 h

Monitor UPC-900

<i>UV unit</i>	
Lamp wavelength	Mercury lamp (for detecting proteins) supplied with 254 and 280 nm filters as standard. Filters for 313, 365, 405, 436, and 546 nm are optional. Zink lamp with 214 nm filter (for detecting peptides) is optional.
Lamp lifetime	Approx. 7000 h (mercury lamp, 254 nm filter, room temp.)
Sensitivity	0.001–5.0 AU
Noise	≤ 4 × 10 ⁻⁵ AU at 254 nm
Flow cell	2- and 5-mm flow cells
Operating temperature	4°C to 40°C
Voltage	100–240 VAC 50–60 Hz
Dimensions (W × H × D)	160 × 200 × 250 mm
<i>Conductivity unit</i>	
Conductivity range	1 µS/cm–999.9 mS/cm
Reproducibility	± 3% or ± 15 µS/cm maximum, short-term
Noise	± 0.5% maximum of full scale
Response time	3 s maximum (0–95% of step)
<i>Flow cell</i>	
Cell constant	50 cm ⁻¹ ± 20
Max. flow rate	100 ml/min
Max. pressure	50 bar (5 MPa, 725 psi)
Cell volume	24 µl
Wetted parts	PTFE and titanium
<i>pH unit</i>	
pH range	0–14 (specifications valid from 2–12)
Accuracy	± 0.1 pH unit, temperature compensated
Stability	0.1 pH unit maximum deviation/10 h
Response time	< 10 s (0–95% of step)
<i>Flow cell</i>	
Max. flow rate	100 ml/min
Max. pressure	5 bar (0.5 MPa, 73 psi)
Cell volume	88 µl
Wetted parts	FFKM, glass, and titanium (flow cell and electrode); PTFE (dummy electrode)

* Measured with water at 1 ml/min, time constant 1 s, 10-mm flow cell.

Fraction collection

With Frac-950 Fraction Collector

Flow rate range	0.001 ml/min–100 ml/min
Fraction capacity	
Optional racks	
Rack A	120 × 18 mm tubes 8 × 30 mm tubes
Rack B	240 × 12 mm tubes
Rack C	Four microplates (4 × 96-wells per plate) 8 × 30 mm tubes
Rack D	45 × 30 mm tubes
Preparative mode ¹	
Rack E	80 × 30 mm tubes
Rack F	20 × 250 mm vessels
Funnel to Flask (used with Rack E)	30 funnels

With Frac-920 Fraction Collector

Tube capacity	95 in Tube Rack 18 mm 40 in Tube Rack 30 mm (optional) 175 in Tube Rack 12 mm (optional)
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¹ Requires Prep Mode Conversion Kit

Sample Pump P-960

Flow rate range	0.1 to 50 ml/min
Operating pressure	0 to 2.0 MPa
Viscosity	Max 5 cP Max 10 cP at reduced flow rate

Autosampler A-900

Sample capacity	96 standard vials (1.5 ml), 160 microvials (0.5 ml)
Injection volume	5 to 1000 µl, flushed loop 1 to 500 µl, partial loop fill 1 to 475 µl, µl pick up
Injection valve switch time	< 100 ms
Precision	
Full loop injections	rsd ≤ 0.3%
Partial loopfill injections	rsd ≤ 0.5% for injection volumes > 5 µl
µl pick-up	rsd ≤ 1.0% for volumes > 5 µl
Sample viscosity	0–10 cP
Programming	Through UNICORN control only
Dimensions (W × H × D)	280 mm × 440 mm × 540 mm

Autosampler A-900 with cooling

Cooling capacity	> 11.5 °C below ambient temperature (T) for 16°C < T < 40°C
Typical cooling times	34 min from 23°C to 4°C (at 45% relative humidity) 60 min from 32°C to 4°C (at 35% relative humidity)
Programming	Through UNICORN control only

Autosampler A-905 for ÄKTA

Sample capacity	
96-well microplate (low/high)	Direct filling or 96 microvials
384-well microplate	Direct filling or 384 microvials
48-vial adapter	48 vials
Injection volume	5–1000 µl, filled loop 1–500 µl, partial loopfill 1–475 µl, µl pick-up
Dispenser syringe	100 µl, 250 µl, 500 µl, and 1000 µl (standard)
Injection precision	≤ 0.3% rsd filled loop > 5 µl ≤ 0.5% rsd partial loopfill > 5 µl ≤ 1.0% rsd µl pick-up
Programming	Through UNICORN only
Cooling capacity	Max. 12°C below ambient temperature for working temperatures between 16°C and 40°C
Dimensions (W × H × D)	280 × 440 × 400 mm

AD-900 A/D Converter

Voltage range	± 2 V DC
Resolution	1 µV, 20 bit
Input impedance	≥ 1 MV
Accuracy	> ± 0.5% or ± 0.05 mV
Max. input voltage	± 5 V
Power requirement	18–36 DC through the UniNet 2 connection
Dimensions incl. attachment (W × H × D)	45 × 85 × 175 mm
Weight	0.4 kg

Computer requirements

Pentium 4, 1.5 GHz
256 Mb RAM
500 Mb available hard disk (150 Mb to run UNICORN), NTFS file system
CD-ROM drive
Windows™ 2000 or XP

Ordering information

Product	Code No.
ÄKTApurifier 10	28-4062-64
ÄKTApurifier 100	28-4062-66
ÄKTApurifier UPC 10	28-4062-68
ÄKTApurifier UPC 100	28-4062-71
Frac-950 Fraction Collector complete with Rack A	18-6083-00
Frac-920 Fraction Collector complete with 18 mm Tube Rack	18-1177-40
Monitor pH/C-900	18-1107-76
Conductivity flow cell	18-1111-05
BufferPrep Kit (includes Valve SV-903 and tubing)	28-4044-73
Buffer selection Kit (includes Valve INV-908, tubing, and inlet filter assembly)	11-0035-96
Column screening Kit (includes 2 Valves PV-908, fitting, and tubing)	11-0035-95
Sample application Kit (includes Pump P-960, Sample pump Kit, Sample valve Kit, and Valve PV-908)	11-0036-00 28-4044-73
Air sensor Kit (includes Air-900 control box, and air sensor Air-925)	11-0035-98
Fractionation Kit (includes Valve PV-908, tubing, and connectors)	11-0035-99
Reverse Flow Kit (includes Valve INV-907, tubing, and connectors)	11-0035-97
Autosampler A-900 with cooling (both include 200 × 1.5 ml sample vials and caps)	18-1144-61
Autosampler A-905 for ÄKTA	18-1175-93
ÄKTAdesign XT Kit* (includes tubing and UNICORN analysis module)	18-6083-19
pH electrode with cell and holder (round tip)	18-1134-84
Dummy electrode, round tip	18-1111-03
ÄKTAextension equipment holder	18-1158-31
Superloop 10 ml (ÄKTAdesign), load 1–10 ml	18-1113-81
Superloop 50 ml (ÄKTAdesign), load 1–50 ml	18-1113-82
Superloop 150 ml, load 1–150 ml (requires Union 1/16" female/M6 male fitting)	18-1023-85
Union 1/16" female/M6 male	18-1112-57

* Required to connect Autosampler A-900 and A-905 to ÄKTApurifier systems

Additional items

UNICORN analysis module	18-1134-74
AD-900 Analog/Digital Converter (includes UniNet cable 0.7 m, Mini-DIN cable 1.5 m)	18-1148-62

Related product literature

Product	Code No.
<i>Data Files</i>	
ÄKTAexplorer™ Systems	18-1124-09
Monitor UV-900	18-1111-17
Pump P-900 series	18-1119-49
Monitor pH/C-900	18-1111-19
UNICORN Control System	18-1111-20
Frac-950 Fraction Collector	18-1153-57
Autosampler A-900	18-1152-89
<i>Application Notes</i>	
Purification of peptides from natural sources	18-1119-52
Efficient isolation of protein fragments for structural analyses	18-1119-53

For contact information for your local office,
please visit, www.gelifesciences.com/contact

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