Science Together



Aruna

P 6.1L

AZURA® Bio purification

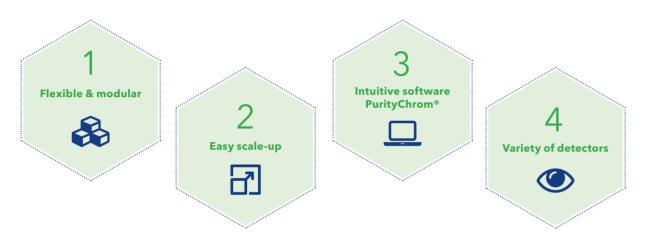
The new standard of FPLC

KNAUER protein purification: The flexible FPLC platform

AZURA[®] Bio purification systems

Complete solutions for FPLC on a minimum foot- The biocompatible/metal free FPLC is the perfect print: AZURA FPLC systems combine flexibility and reliability.

choice for your protein purification task.



Design your AZURA Bio purification system to your needs. Multiple functionalities such as automatic sample injection via autosampler, column switching, buffer and sample selection as well as fraction collection enable the user to automate the purification process.

A large range of different detectors make your target molecules visible. Different flow rates and compatibility to columns from all venders offer maximum flexibility. The intuitive software PurityChrom[®] combines all the advantages of a versatile purification software.

Fast Protein Liquid Chromatography (FPLC)

FPLC is a form of liquid chromatography to purify large biomolecules like proteins or DNA. External factors like high temperature, high pressure, extreme pH, or solvents can disturb the protein structure and are therefore avoided in FPLC. Besides,

the method uses column materials out of agarose or polymer material which are very sensitive against pressure fluctuations and air bubbles. We designed our systems to meet your purification challenges!

AZURA® Bio purification: You choose the method

Size Exclusion Chromatography (SEC)

Affinity Chromatography (AC)



Separate according to size. See page 18 for a specialized AZURA system for SEC.

Specific binding of protein of interest. See page 19 for a specialized AZURA system for AC.

Purification strategy: Often a sequence of different methods is used in purification.

Capture	> Intermed	Jiate
Normally a combination of me	thods is used in pro	otein purification.

- The "capture" step purifies the protein from the crude extract.
- The "intermediate" step removes further contamination.
- The aim of the final "polishing" step is to get rid of all remaining impurities
- in order to gain a highly purified product.

Continuous FPLC purification for more productivity?

Use AZURA SMB as your step to the future of biopurification and run your downstream process with the highest productivity and lowest costs. Our multi-column capture process (MCCP) saves your time as well as solvent and column costs.

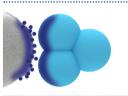
For more information see page 26.

Ion-Exchange Chromatography (IEX)



Separation takes place according to the charge of the protein and gradient elution.

Hydrophobic Interaction Chromatography (HIC)



Separation is performed based on hydrophobic interaction and gradient elution.

Polishina



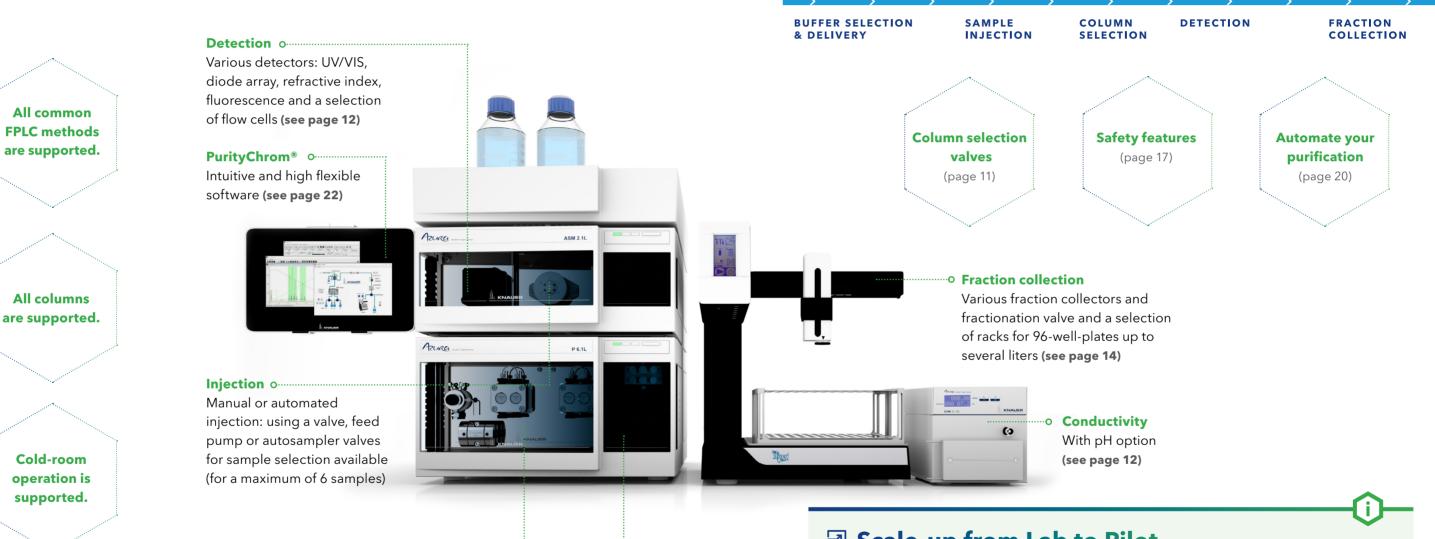
3

AZURA® Bio Lab

From simple to complex, from Lab to Pilot scale: Design your AZURA® FPLC system according to your purification task!

AZURA Bio Lab allows you to create FPLC systems ules and build-up the system yourself. Continue with highest independence. Just pick your mod-

flexibility with intuitive PurityChrom[®] software.



Buffer delivery o Quaternary, binary pumps

with flow rates up to 10 ml/min or 50 ml/min (see page 6)

Buffer selection o Integrated buffer selection valve for 4 buffers, extra buffer selection valves available (see page 6)

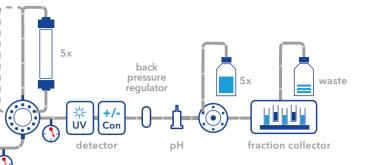
Scale-up from Lab to Pilot Choose the Pilot series if you want to increase your productivity **Configure your AZURA Bio system** even more. Upscale our Lab configuration with same flexibility, software PurityChrom[®] but minimal footprint. Just transfer and upscale Find all FPLC products on the your methods. Flow rates up to 1000 ml/min and loads up to sevfollowing pages.

eral grams are possible. Find more information: www.knauer.net

0

0 sampl

0



5

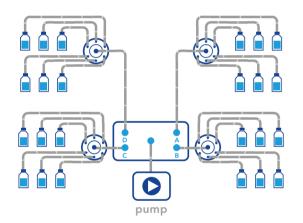
Buffer delivery

Precise and reliable pumps covering a wide flow rate range, gradient and Buffer selection options.

Buffer selection

Automated switching between buffers is important for method development, column cleaning and regeneration. The pump P 6.1L features a build-in 2 x 2 buffer selection valve (A1, A2 and B1, B2) or 4 x buffer selection low gradient valve (A, B, C, D).

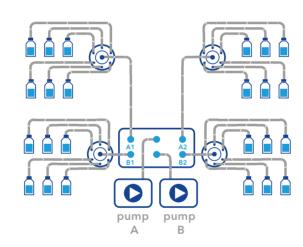
You can extend buffer selection with additional valves each for 6 buffers.



AZURA pump P 6.1L LPG - Quaternary gradient

Compact pump AZURA® Pump P 4.1S

Isocratic pump with small footprint for dedicated applications or sample loading.



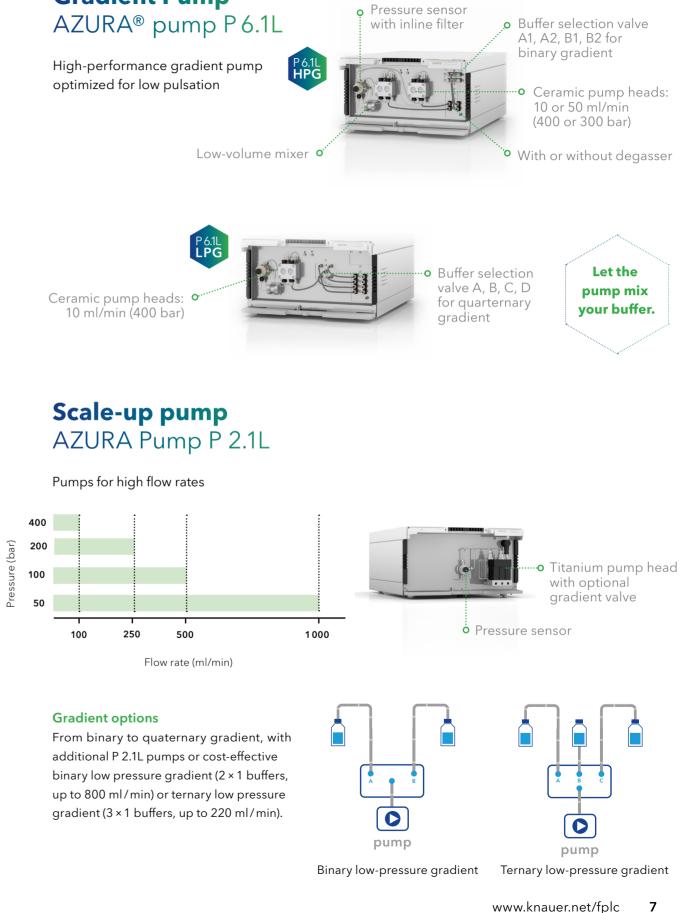
AZURA pump P 6.1L HPG - Binary gradient



Gradient Pump



10 ml/min (400 bar)







Binary or quaternary gradient?

A quaternary low pressure gradient (LPG) module* dynamically composes the buffer on the inlet-side or low pressure side of the pump head, by guickly switching the selection valve between the dif-

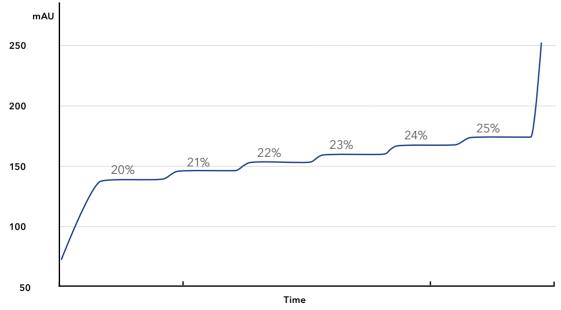
ferent channels. The buffer in a binary gradient (HPG) system is composed by combining the solvent flows of two isocratic pumps.

Quaternary gradient

- Low investment costs
- Limited flow rate range
- Channel usable for sample injection
- Gradient accuracy absolutely sufficient for FPLC

Binary gradient

- Less wear ٠
- No flow rate limitation •
- Sample pump for sample injection
- High accuracy for special application



Excellent gradient reproducibility of 0.3 % RSD. Overlay of 6 repetitions at 1 ml/min run with pump P 6.1L low pressure gradient version

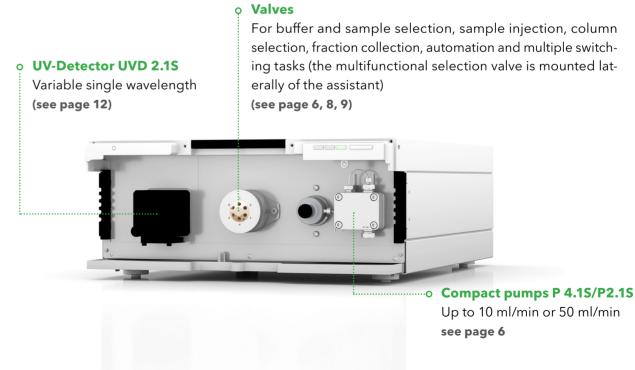
AZURA® ASM 2.1L Assistant

A flexible combination module

modules the assistant fulfills many different tasks The Assistant ASM 2.1L is a compact combination module which can be equipped with up to three like sample and buffer selection, sample injection, device modules. Available for selection are valves, column switching, fraction collection, buffer depumps, and a UV detector. An assistant including livery or UV-detection. a pump, valve, and detector features a compact The concept of the flexible combination of device FPLC system, like AZURA Bio SEC or AZURA Bio AC. As a part of a larger system, the ASM 2.1L is modules combines the highest functionality with extremely versatile. Depending on the integrated minimal space requirements.

Configure your assistant

Can be equipped with combinations: valves, pumps, and one detector



For detailed information on device modules and assistant configuration: www.knauer.net

9

Sample injection

Available sample injection modules range from an injection valve, sample pump and autosampler.

Injection valve

Integrated into assistant or standalone module: The AZURA 2-positions valve is perfect for injection of small sample volumes. Connect 1/16" tubings for flowrates up to 100 ml/min. For higher flowrates use the injection valve for 1/8" tubing. Various sample loops are available.

Sample pump

Integrated into assistant or standalone module: The AZURA P 4.1S is perfect for injection of larger sample volumes.

Repetitive sample injections by using the pump for automated sample loop filling.

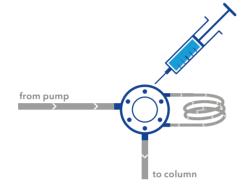
Do vou have many samples?

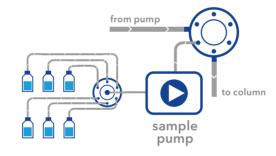
You can extend your configuration with additional valves each for 6 samples.

Autosampler

Process many different samples fully automatically with the Autosampler AS 6.1L.

- Up to 10 ml injection volume
- From microtiter plates to standard vials
- Active cooling
- Fully supported by PurityChrom[®] software
- Metal free







Column selection

Different options for column selection are available.

2-position valve

- Select two columns or one column and one bypass
- Flow rates up to 500 ml/min possible

Multifunctional selection valve

- For up to 5 columns and 1 bypass
- Reverse flow
- Flow rates up to 50 ml/min

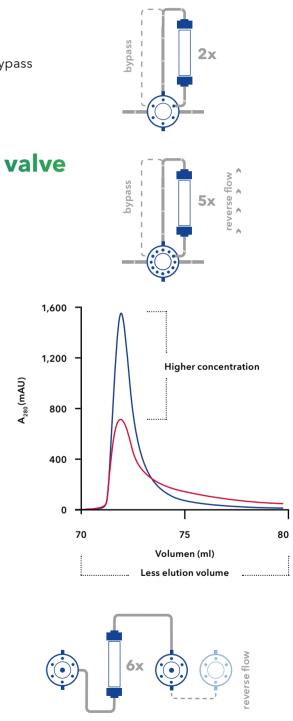
Why is the reversed flow option popular in affinity chromatography?

In affinity chromatography your target molecules will accumulate at the top of the column. Elution in the same direction dilutes your target molecule along the column. By elution with reversed flow you increase the concentration while decreasing the sample volume.

This option has two major advantages. Clean your columns more efficiently using reverse flow. By this you elute contamination the shortest way and minimize damage to the column.

Higher flow rates?

Use the column selection assistant to select six columns assuring a flow rate up to 500 ml/min. An additional valve allows to reverse the flow.



AZURA® UV Detectors

We provide a choice of UV/VIS detectors, ranging from single variable wavelength to 8-channel diode array detector with 3D scan capability.



UVD 2.1S	MWD 2.1L	DAD 2.1L
Compact and cost-ef- fective variable single wavelength UV/VIS detector	Reliable multichannel UV/VIS detector	Diode array detector for peak purity check
190-500 nm	190-700 nm	190-700 nm
1	4	8
n/a	n/a	+
+		
	Compact and cost-ef- fective variable single wavelength UV/VIS detector 190-500 nm 1 n/a	Compact and cost-ef- fective variable single wavelength UV/VIS detectorReliable multichannel UV/VIS detector190-500 nm190-700 nm14

More UV detectors available for your applications: www.knauer.net/detectors



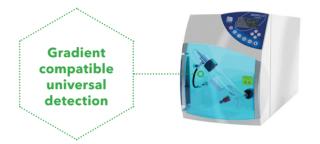
AZURA® CM 2.1S

- Conductivity monitor for checking salt gradient
- Flow rates up to 100 ml/min
- 0,01 mS/cm-999 mS/cm
- pH option available

Flow cells for CM 2.1S				
 Analytical	1/16"	10 ml/min	160 bar	30 µl volume
Preparative	1/16"	100 ml/min	100 bar	300 µl volume



A wide range of third-party detectors can be seamlessly integrated into AZURA® systems.



Fluorescence detector RF-20A

The fluorescence detector RF-20A provides world-class sensitivity, excellent maintainability and diverse validation / support functions. It supports a wide range of applications from conventional to high-performance analysis.



The KNAUER interface box IFU 2.1 LAN allows highly precise analog data acquisition of third party modules over analog and relay outputs. Example: MALS-detectors for molecular weight determination.

Flow cells

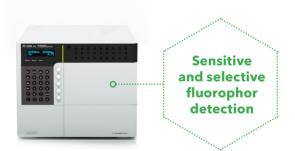
Select from an impressive range of easily exchangeable flow cells which cover a wide range of application. Optional fiber optics technology offers the possibility to separate the flow cell spatially from the device providing enhanced security for hazardous, explosive or toxic work processes.

AZURA® RID 2.L

Refractive Index Detector for cost-effective, fast and reliable analysis of non-UV absorbent compounds.

SEDEX-LC

Using the unique Low Temperature technology, this Evaporative Light Scattering detector LC allows universal high sensitivity detection of non-UV active substances.



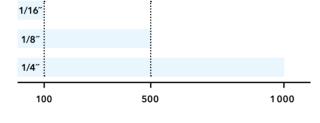
Fraction collection

Collect large quantities or large numbers of fractions

Manually - collection by direct control Volume-based - collection at defined volumes Peak-based - collection according to detector signal

Fraction valves

- Collecting large quantities
- Up to five fractions and waste
- Available as a single device or integrated into an Assistant ASM 2.1L for different flow rates



Fractionation valves max. flow rate (in ml/min)



Foxy Fraction collector

The Foxy R1 and Foxy R2 are versatile fraction collectors which fits to every purification need.

- Foxy R2
- Wide choice of racks from 96-well microplates up to bottles or funnels
- Double capacity for Foxy R2 with automatic rack recognition
- Active cooling for Foxy R1
- Supported in software Puritychrom[®]
- Stand-alone operation
- Repeated collection in same vials



Vario 4000 & Vario 4000 plus

The Vario 4000 is a more advanced fraction collector for demanding applications with high flow rates and a high number of fractions. Indivi-• Up to 125 ml/min for Foxy R1 and 1000 ml for dual rack types are programmable. Just assemble your rack to your needs.

- For flow rates up to 1000 ml/min
- High number of fractions
- Supported in software Puritychrom[®]
- Standalone operation

Accessories

Accessory	Features	Benefit
Pressure Control	 Contains two pressure sensors Automatic determination of pressure difference with Purity-Chrom[®] Connect 1/16" or 1/8" tubings Up to 250 ml/min and 60 bar 	Monitor pressure over the column bed and protect column from damage
Air Sensor	 Detect end of buffer or end of sample with PurityChrom[®] Up to four air sensors per system For transparent tubings with 1/16" or 1/8" or 1/4" outer diameter 	Protect column from air damage and support automation (e.g. sample injection)
AZURA Click	 Attach air sensor, pressure con- trol, AZURA Organizer or your interface box to the side panel of your AZURA L device 	Organize your system.
AZURA Organizer	 Attach columns from 5 mm to 26 mm diameter, falcon tubes, a back pressure regulator or a pH flow cell 	Organize accessories direct at the system and reduce dead volume
Back pressure regulator (BPR)	 Apply a constant back pressure to your system Freely adjustable between 1-20 bar or 20-103 bar 	Prevent formation of air bubbles after the column which disturb detector signa

AZURA® Bio purification systems

Product	Features	Page
AZURA Bio SEC	0.001-10 ml/min, maximum 200 bar, injection valve sample for sample loops, variable single wavelength UV-detector, XY fraction collector, PurityChrom® software	18
AZURA Bio AC	0.01-50 ml/min, maximum 200 bar, selection valve for 6 buffers/samples, variable single wavelength UV-detector, fraction valve for 5 fractions and waste, PurityChrom® software	19
AZURA Lab LC	0.001-50 ml/min, maximum 200 bar, injection valve sample for sample loops, variable single wavelength UV-detector, XY fraction collector, PurityChrom® software in basic configuration. Configure your FPLC system based on your purification requirement.	4
AZURA Bio Lab Two-step purification	0.01 - 50 ml/min, maximum 200 bar, sample injection via sample loop or sample pump, automated storage and reinjection of proteins, variable single wavelength UV-detector, XY fraction collector, PurityChrom® software	20
AZURA Bio Pilot	Up to 1000 ml/min, sample pump for large sample vol- umes, variable single wavelength UV-detector, XY fraction collector, PurityChrom® software in basic configuration. Configure your FPLC system based on your purification requirements. Scale-up is possible with same flexibility, software but minimal footprint.	5

Components from Lab to Pilot

I	
Product	Features
Buffer Delivery	
Compact pump	10 or 50 ml/min, isc
Gradient pump	10 or 50 ml/min, Qu C, D) Binary: selection
Scale-up pump	100, 250, 500, 1000
Extended buffer selection	With additional valv
Sample selection	For maximum 6 sam
Column Selection	
Column selection valves	For 2 columns, 5 col option
Detection	
Wide choice of detec- tors	Variable single wave Full spectra diode a monitor, Fluorescen
Fraction Collection	
Fractionation valve	For five fractions an
Fraction collector	From 96-well microp up to 1000 ml/min
Sample Injection	
Injection valve	1/16" tubing: up to 50
Sample pump	10 or 50 ml/min
Autosampler	Up to 10 ml injection standard vials
Software	
PurityChrom software	Highly flexible meth volume- or time-bas visualisation, hold & functions, Check for
Safety Features	
Accessories for protec- tion and automation	Air sensor, Pressure management, mour

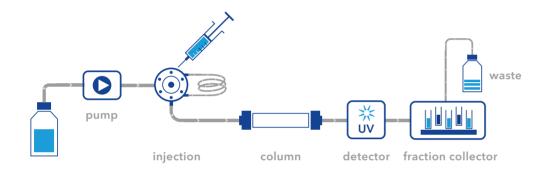
	Page
ocratic	6
uaternary: selection of 4 buffers (A, B, ion of 2 buffers (A1, A2, B1, B2)	7
) ml/min, Binary to quaternary gradient	7
ves each for 6 buffers	6
nples	10
lumns and 5 columns with reverse flow	11
elength UV, Multiple wavelength UV, array (DAD), Conductivity and pH nce, Refrective Index	12
nd waste, up to 1000 ml/min	14
plates up to bottles or funnels,	14
0 ml/min 1/8" tubing: up to 500 ml/min	8
	8
on volume, from microtiter plates to	8
nod writing, intuitive user-interface, sed, with special features like system & adjust option, extended threshold r impurities	21
e Control, Back-pressure regulator, leak nting solutions	14

AZURA® Bio SEC

Time consuming gelfiltation runs?

AZURA Compact SEC systems take over time-consuming SEC methods in your lab without blocking your valuable FPLC system. Thanks to its compact design and intuitive FPLC software PurityChrom[®], the system offers outstanding performance and

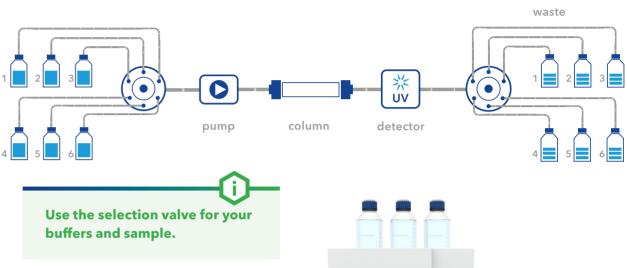
ease of use. Pre-designed methods are included in the software and can be easily adapted by changing the column volume. AZURA Compact SEC supports all columns available on the market.



AZURA® Bio AC

For affinity chromatography

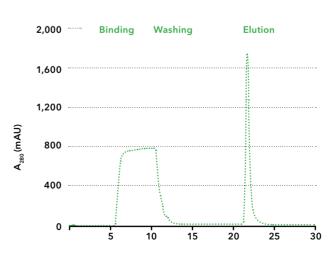
The AZURA Compact AC system qualifies for fast and reliable affinity chromatography. Select your sample, your washing and elution buffer using





Key features

- Flow rate: 0.001-10 ml/min; 0.1-8.0 ml/min (recommended)
- Maximum system pressure: 150 bar
- Injection valve for sample injection via sample loop
- Variable single wavelength UV-detector (190-500 nm)
- Fraction collector for fractionation
- Columns from all venders can be used
- PurityChrom[®] software





the selection valve. Your proteins of interest are detected by UV and automatically collected via the fractionation valve.



Key features

- Automatic sample/ buffer selection valve for up to 6 buffers or samples
- Fraction valve (6 ports) for fractionation
- Flow rate: 0.01-50 ml/min; 1-40 ml/min (recommended)
- Variable single wavelength UV-detector (190-500 nm)
- Columns from all venders can be used
- PurityChrom[®] software
- Maximum system pressure: 150 bar

Capture proteins based on high affinity Chromatogram & Legend

Special configuration -Two step purification

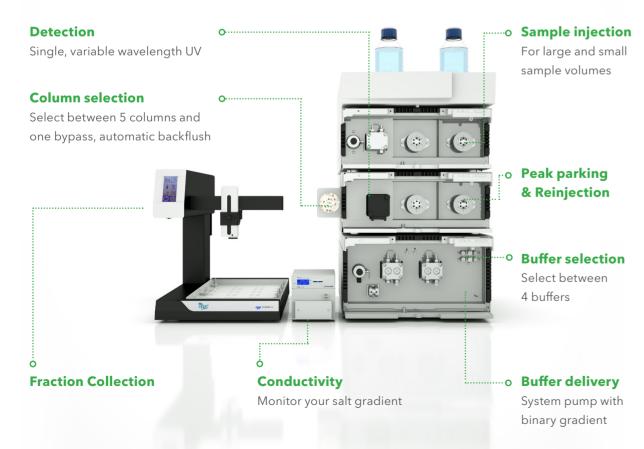
Special multi-column chromatography solutions

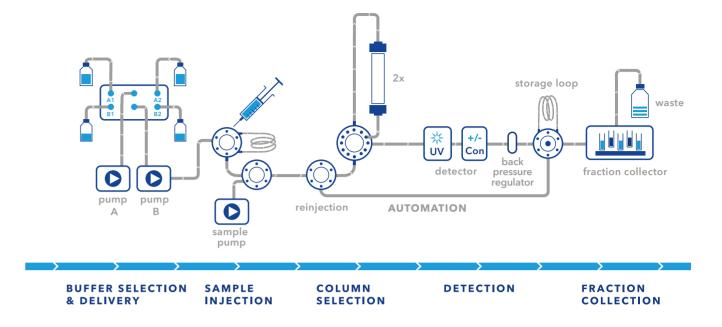
Protein purification involves most of the times two to three steps:

- 1. capture step
- 2. optional intermediated step
- 3. polishing step

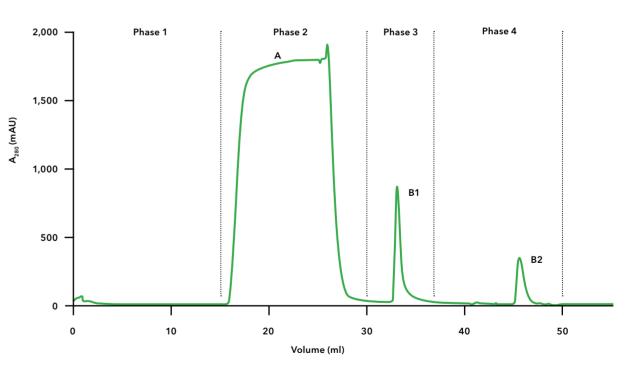
The transition from one to another step generally involves manual interaction and thus is time consuming. Automation by combining these steps increases the efficiency and optimizes the workflow. The quick and automated linkage of multiple

chromatographic purification steps into one method eliminates manual sample handling and minimizes time spent between steps. This automation strategy can be easily adapted to each purification task.





Automated two-step purification of mouse IgG antibodies

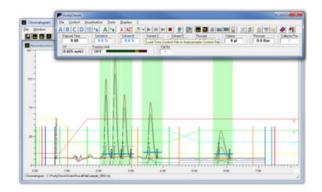


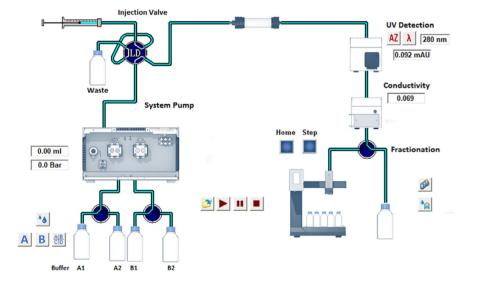
The affinity chromatography step was automatically combined with a gel filtration step to exchange the buffer of the purified mouse IgG antibodies; Phase 1: Column equilibration, Phase 2: Sample injection and washing, Phase 3: Elution of IgG from protein A column, Phase 4: Desalting of IgG

Control your purification

PurityChrom

PurityChrom is a powerful software to control your FPLC system. Get familiar with PurityChrom in shortest time and with no effort due to the intuitive and clearly structured user interface. Choose a time-or volume based workflow by just clicking one button. Create methods with highest flexibility to realize complex application without losing easy handling. Offline licenses for creating methods and data evaluation are for free.





System Visualisation

Keep an eye on your system with the system visualisation. The interactive flow path allows to control your system. Switch valves, start pumps, set autozero, start fraction collection.

Hold & Adjust (a running method)

You have full control of your run. Hold a run to adjust the method or the system. Stay always in control and change the parameters of a running method.



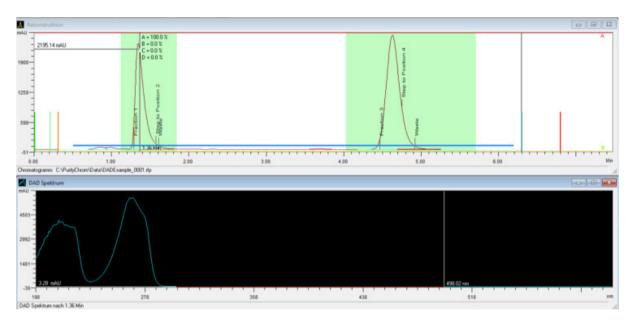
Extended threshold functions

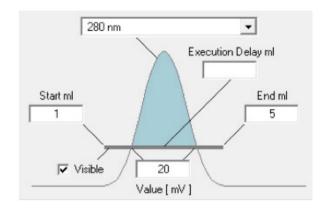
Automate any software function triggered by signals of any channel.

Automatically start fraction collection at the beginning of your desired peak. Protect the system from overpressure and air bubbles. After end of sample detection the software offers the possibility to automatically start or continue the run. Automate the whole purification starting from sample injection, via column washing to elution.

Check for impurities full spectra diode array (DAD)

Check the purity of your peaks based on the absorbance spectra anywhere in the elution profile.





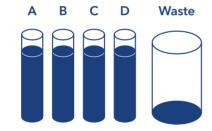
Intuitive data analysis

Integrate peaks fully automatically or manually. Receive the peak results by click on one button.

Integration Tools Automatic Integratio 1 1 Maximum Baseline Level : 250 [mV] Filter Factor : 5 [Slices] Slone Sensitivity ...: 300 [µV/sec] Manual Integration *

Solvent Supply - Calculate the consumption of buffers

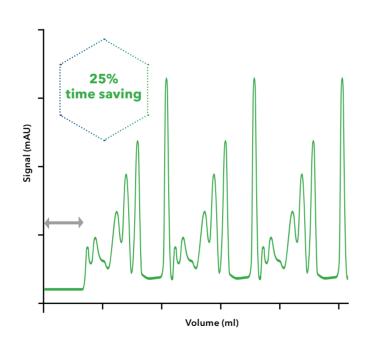
The solvent supply function calculates the consumption of buffers and the waste level for the current run, thus preventing the column from running dry and flooding the lab.



Stacked Injection

Size exclusion chromatography separates the proteins according to their size. After selection of SEC medium, sample volume and column dimensions are the two most critical parameters that will affect the resolution of the separation. For most SEC runs the sample volume should not exceed 2% of the total column volume to achieve maximum resolution.

For larger sample volumes the sample must therefore be divided into different runs. However, this takes a lot of time and is not very efficient. With the stacked injection function in PurityChrom it is possible to run different runs automatically one after the other. The injection of the next run takes place during the current run, so that the time until the elution of the first peak can be fully exploited. This increases efficiency and saves time.



Customer review

AZURA[®] Bio purification solution by KNAUER.

"Our KNAUER FPLCs are the workhorses in the lab."

"My lab studies the structure and function of membrane proteins. Due to the inherent instability of these proteins we purify them in the cold room. We needed robust FPLCs with good pumps that tolerated these conditions well.

In addition, the systems needed to be easy to maintain. Knauer provided us with skilled advice on virtually every component of the system, ranging from tubing and pumps up to the software. Consequently, our systems are perfectly tailored to our needs. Most of the maintenance we can do ourselves. For remaining questions, we can rely on the great support Knauer offers. Our Knauer FPLCs are the nononsense workhorses in the lab. I highly recommend Knauer."







Jun. Prof. Dr. Eric R. Geertsma Institute of Biochemistry, Goethe-University Frankfurt Foto: Uwe Dettmar

System components

- AZURA[®] UV Detector UVD 2.1S
- AZURA[®] Valve Drive V 2.1S
- AZURA[®] Pump P 4.1S
- Foxy fraction collector



AZURA Compact SEC systems take over time-consuming SEC methods in your lab without blocking your valuable FPLC system.

Contact us: sales@knauer.net

AZURA® Multicolumn



Continuous separation of biomolecules

The flexible design of the AZURA® SMB system is perfect continuous FPLC purification. Use AZURA® SMB as your step to the future of biopurification and run your downstream process with the highest productivity and lowest costs. Our multi-column capture process (MCCP) saves your time as well as solvent and column costs.

Multi-column capture processes (MCCP)

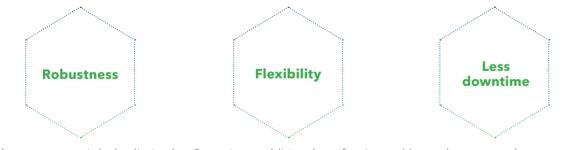
Continuous IEX or affinity chromatography is almost as simple as a standard batch run. Depending on your batch separation, you can easily configure the parameter for the continuous run. Thanks to the system flexibility it's very easy to change the column distribution for binding (d), washing (c), eluting (b) and regeneration (a) of the columns. If the washing steps take longer, just use more columns in this zone.

Why an 8 column setup is the best

Three reasons why more columns are better

The separation of biomolecules can also be done with 2, 3 or even 4 columns. But these systems are often very limited and cannot handle typical

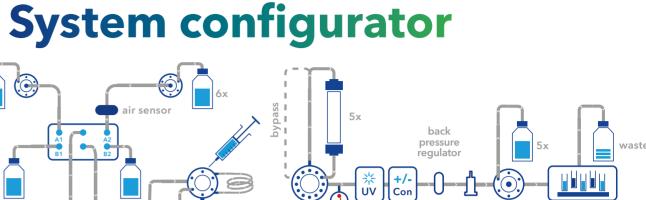
problems regarding process stability and flexibility as easy as the AZURA® SMB system can do. The 8 column setup enables many advantages.

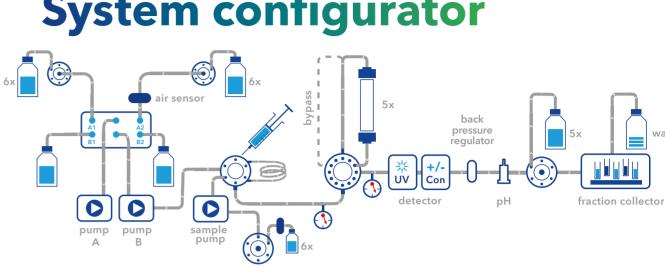


Other systems might be limited regarding their batch program adaption (wash+elution time \leq capture time), due to the flexible column distribution the length of every zone can be adjusted, even if there is a critical change in the feed concentration.

Sometimes additional purification steps are necessary to regenerate the column and keep their lifetime and potential at a maximum. Two and three columns systems often cannot handle this problem easily.

More columns mean longer system run time and less downtime. You will have up to four times less downtime due to column change. Use your working time most efficient.





BUFFER SELECTION & DELIVERY

10 ml/min binary gradient pump P6.1L	Injection valve
gradient panip route	Quantity
50 ml/min binary	Sample pump
gradient pump P6.1L	module
□ 10 ml/min quaternary	Quantity
pump P6.1L	
□ 100 ml/min pump P2.1L	 Sample selection valve (6 further inlets)
Quantity	Quantity
250 ml/min pump P2.1L	Biocompatible Autosampler AS6.1L
Quantity	Autosampier A30.1L
500 ml/min pump P2.1L	
Quantity	
,	
□ 1000 ml/min pump P2.1L	
Quantity	
Ternary gradient	
module for pump P2.1L	
Binary gradient module for pump P2.1L	
 Buffer selection valve (6 further inlets) 	
Quantity	
,	

SAMPLE

INJECTION

COLUMN SELECTION

- □ Column selection valve up to 50 ml/min (5 columns, one bypass, reverse flow)
- □ Column selection (two columns or one bypass)
- □ Column selection high flow (5 columns, one bypass)
- □ Column selection high flow (5 columns, one bypass, reverse flow)

DETECTION

- single wavelength
- multiwavelength
- □ Conductivity
- 🛛 pH
- □ Fluorescence
- □ Refrective index
- □ Light Scattering
- 🗆 Analog integration of further detectors

FRACTION COLLECTION

□ Fractionation valve

□ Foxy fraction collector with fixed rack types

Labocol fraction collector with individual rack types

ACCESSOIRES

- □ Airsensor 1/16″
-Quantity
- □ Airsensor 1/8″
-Quantity

Pressure control (2 pressure sensors)

Back pressure regulatorQuantity

AZURA OrganizerQuantity

Analytical HPLC

Multi-Column Chromatography, SMB

Preparative HPLC

FPLC

Osmometry

Dosing, Metering, Pumping

Detection

Technical data or prices are subject to change without notice. Prices may vary by country and do not include taxes, customs duties or delivery.

Distributed by:

All trademarks are the property of their respective owners. Our general terms and conditions apply: www.knauer.net/terms

KNAUER

Wissenschaftliche Geräte GmbH Hegauer Weg 38 14163 Berlin, Germany +49 30 809727-0 +49 30 8015010 (Fax) info@knauer.net www.knauer.net

