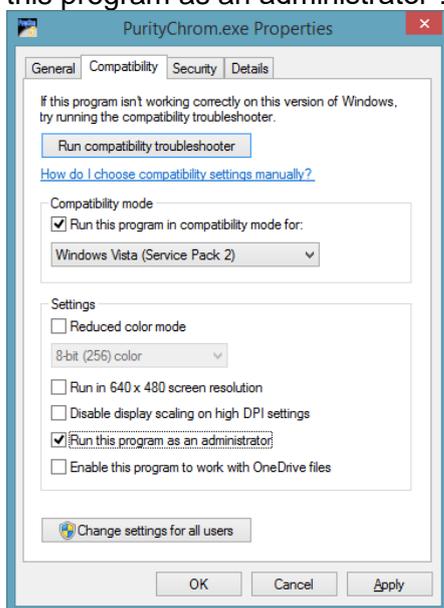


Installation Guide PurityChrom®

1. PurityChrom operates under Microsoft Windows XP, Windows Vista, Windows 7, Windows 8 and Windows 10.
The supported languages are: german and english
2. Automatic switching to energy saving mode or hibernating has to be disabled i.e. settings for “Turn Off Hardware”, “Turn off Computer” or “System Standby” must be set to “Never” in the control panels energy options. Microsoft Security Essentials is installed, please exclude PurityChrom. Therefore, open Microsoft Security Essentials and go to Setup. In “Excluded files and locations” you have to search for C:\PurityChrom and add this folder to excluded files. In “Excluded processes” you have to search for C:\PurityChrom\PurityChrom.exe and add this process to the excluded processes. Furthermore, the Windows Defender has to be deactivated. Therefore, got to “Extras” and/or “Settings”. Choose than “Administrator” and uncheck “Turn on this program” or Turn on this app”.
3. The user account settings have to be set to “Never notify” before installation. After the installation, the settings can be reset. (Start → Control Panel → User Accounts → User Accounts → Change User Account Control Settings → Never notify)
4. Insert the CD which includes the PurityChrom.
5. Start the installation as administrator. The installer will ask you to choose the language of the software, your integrated UV-detector and the destination location for the files that will be installed. Do not change the given destination.
6. Restart your computer.
7. Installation under Windows 8 or 10: Go to C:\PurityChrom and right-click the PurityChrom.exe to open the properties. Go to compatibility and check “Run this program in compatibility mode for” and choose “Windows Vista (Service Pack 2). After this, check “Run this program as an administrator“. Click ”Apply“ and ”Ok“, than close the window.



8. Connect your devices. All devices that should be connected by LAN should be set to LAN and need fixed IP addresses.

AZURA L	Mobile Control/Service Tool/Firmware Wizard (in folder "Tools" on PurityChrom CD)
UVD 2.1S	Display/Firmware Wizard (in folder "Tools" on PurityChrom CD)
AZURA S	Service Note 0106/Display
Conductivity Monitor CM 2.1S	ConfigTool107 (in folder "Tools" on PurityChrom CD)
Foxy R1/R2	Display
Autosampler AS 6.1L	Service Note 0153 - Point 3
LABOCOL Vario 4000	Internet browser (type in IP 192.168.0.112), more information in device manual

9. Sensing of leaks is not supported in PurityChrom. Deactivate the leak sensor of AZURA L devices.

10. The KNAUER Interface Box IFU 2.1 requires a separate driver for the built-in USB-to-serial controller. If no VSCOM USBtoCOM box is installed, you can install the driver from the PurityChrom CD, folder:
Drivers\COM Extensions\VSCOM USBtoCOM\Version_2.08.28_Win7, XP, 2003, x86_x64.
The file must be executed with administrator access.

*For Windows 8.1 please use the IFU 2.1 driver from the following folder:
Drivers\COM Extensions\VSCOM USBtoCOM\Version_2.08.30_81_Win8.1*

11. The KNAUER RID 2.1L and Shimdazu fluorescence detector require a separate driver for the built-in USB-to-serial controller. Copy the driver from C:\Windows\PurityChrom\DriverDll:
RID21L.dll rename to: PrepconLF.dll (delete original PrepconLF.dll before)
RF20A.dll rename to: PrepConAddCh.dll (delete original PrepConAddCh.dll before)
and copy them to C:\Windows\System32 (32-bit system) or C:\Windows\SysWOW64 (64-bit system)
12. In the next step, you have to change the "PurityChrom.ini"-file according to the connected devices. This file is located in C:\Windows\

WinsockPorts

- In [WinsockPorts] the IP-addresses and Ports of LAN connected devices are entered.
For example: Port1=192.168.5.1,10001
(freestanding devices have the port number 10001,
the left module in an assistant has the port number 10002,
the module in the middle the port number 10003 and
the right module the port number 10004.
Port for Autosampler KNAUER 3950: 2101
Port for Fraction Collector Foxy R1/R2: 23
- If the assistant contains only one module, it is always connected to the left port (IP port 10002) despite of the module position.
- Devices which are connected via serial interface are not filled in.

P4.1S/P2.1S (high pressure gradient system in one assistant)

- Enter the port of both pumps under one entry under [WinsockPorts]. For example
Port1=172.31.5.25,10002,10003
- There is a separate entry for pumps parameter under [Pumps].
→ Set "SoftwareHPG=0" (for isocratic) or "SoftwareHPG=2" (for binary gradient)
below "PreciseMixing=0"

High pressure gradient system (independent pumps)

- Setting for pumps: isocratic
- Enter the IP address and the port of pump A under [WinsockPorts].
- There is a separate entry for pumps parameter under [Pumps].
Set:
SoftwareHPG=1 for isocratic,
SoftwareHPG=2 for binary gradient
SoftwareHPG=3 for ternary gradient or
SoftwareHPG=4 for quaternary gradient

below "PreciseMixing=0"

→ edit lines for pump B, C and D. For example:

```
SoftwareHPGPort2=127.0.0.2,10001  
SoftwareHPGPort3=127.0.0.3,10001  
SoftwareHPGPort4=127.0.0.4,10001
```

with the IP-Address and Port of Pump B, C and D.

Low pressure gradient system (P 2.1L)

- Insert in Puritychrom.ini the following entries:
[Pump]
LPGType=2 (for binary LPG)
or
LPGType=3 (for ternary LPG)

Using a Triggersignal

- Change the Puritychrom.ini file as described here:

```
[Pumps]
IOControl=1
```

Constant Pressure Mode

- To use the constant pressure mode change the Puritychrom.ini file as described here:

```
[Pumps]
ConstantModeMajor=1
```

Pumps do not stop at command "Stop all"

- You can decide whether pumps should stop or pump further when pressing "Stop all".

```
[Pumps]
PumpStopRequest=1
```

UV-Detector

- There is a separate entry for the connected detector in the PurityChrom.ini-file:

```
[KnauerUVD21S]
Enabled=0          [ 0/1 ]      OFF = 0, ON = 1 (using a detector
                           connected via serial interface or via LAN the
                           value has to be set to 1; using a detector
                           connected via an A/D converter the value
                           has to be set to 0)

IP=                [ IP ]      IP-address
Port=              [ Port ]    port number (KNAUER: 10001)
BaudRate=9600     [ Value ]    baud rate
Wavelength1=254   [ Value ]    preset wavelength
DataRate=2        [ Value ]    Datarate of the detector (don't change the
                           data rate as the detector will send the
                           maximum data rate)

Channels=1        [ Value ]    Number of channels
MaxD2Time=0       [ 0 - 2000 ]  0=off, 1-2000 = Hours;
                           When the maximum value is exceeded, an
                           error message occurs. However, work may
                           be continued after confirming the message

Type=UVD2.1S     [ Type]      Your detector type (UVD2.1S, UVD2.1L,
                           50D, MWD2.1L)
```

- If the UV-detector is connected via serial interface hide the IP address by using an inverted comma ('IP=) and enter the number of the COMPort under Port.

DAD

- There are two separate entries for connecting a DAD in the PurityChrom.ini-file:

[KnauerDAD61L]

Enabled	[0/1]	OFF = 0, ON = 1
IP=	[IP]	IP-address
Port=	[Port]	port number (KNAUER: 10001)
Integration=100	[Value]	Preset value for DAD integration time.

[DAD]

Enabled=0	[0/1]	OFF = 0, ON = 1
Diodes=1024	[Value]	Number of diodes in the diode array. 1024 = DAD 6.1L / 256 = DAD 2.1L
ScanStart=200	[Value]	Spectrum start of ECOM-DAD.
ScanEnd=600	[Value]	Spectrum end of ECOM-DAD.
SliceWidth=100	[Value]	Preset value for DAD slicewidth.
IntegrationTime=100	[Value]	Preset value for DAD integration time.
IntegrationBandwidth=1	[Value]	Preset value for DAD integration bandwidth.
AutomaticReference=1	[0/1]	Automatic reference spectrum after starting a time control file.
LambertBeer=1	[Value]	This value is required. Do not change!

Conductivity Monitor

- There is a separate entry for the connected conductivity monitor in the PurityChrom.ini-file:

[KnauerCM21S]

Enabled=0	[0/1]	OFF = 0, ON = 1 (using a detector connected via serial interface or via LAN the value has to be set to 1; using a detector connected via an A/D converter the value has to be set to 0)
IP=	[IP]	IP-address
Port=	[Port]	Port number (KNAUER: 10001)
DataRate=1	[Value]	Datarate of the monitor
Channels=1/2	[Value]	Number of channels (1 = conductivity, 2 = conductivity and pH)

- If the conductivity monitor is connected via serial interface hide the IP address by using an inverted comma ('IP=) and enter the number of the COMPort under Port.

Fluorescence Detector

- There is a separate entry for the connected fluorescence detector RF20A in the PurityChrom.ini-file:

```
[ShimadzuRF20A]
Enabled=          [ 0/1 ]    OFF = 0, ON = 1
Port=             [ Port ]    Port number
Channels=1        [ Value ]    Number of channels
Gain=1            [ 1/2/3 ]    1=x1, 2=x4, 3=x16
Sensitivity=0     [ 1/2/3 ]    0=High, 1=Medium, 2=Low
DataRate=2        [ Value ]    Datarate of the detector
AnswerTimeOut=1  [ Value ]    Do no change
ConnectionTimeOut=1 [ Value ]    Do not change
Output=2          [Value]     0: Excitation energy, 1: Light source
                                correction, 2: Emission not corrected
```

Do not use USB-to-COM adapter to connect the Shimadzu fluorescence detector.
Settings for Shimadzu RF20A:
Baudrate: 9600, Data Bits 8, Parity 0, Stop Bit 1

RI Detector

- There is a separate entry for the connected RID 2.1L in the PurityChrom.ini-file:

```
[KnauerRID21L]
Enabled=          [ 0/1 ]    OFF = 0, ON = 1
IP=              [ IP ]     IP-address
Port=            [ Port ]    Port number (KNAUER: 10001)
DataRate=        [ Value ]    Datarate of the detector
Channels=1        [ Value ]    Number of channels
TimeConst=2000   [ Value ]    Time constant
SignalMode=0     [ Value ]    Signal mode, 0=direct, 1=inverted
AnswerTimeOut=1  [ Value ]    Do no change
ConnectionTimeOut=1 [ Value ]    Do not change
Temperature=0 / 30-55 [ Value ]    0 = off, 30-55 = Temperature is set after
                                start
TempZone=1       [ 1-8 ]    1= [Temperature] FunctionName1=...
                                2= [Temperature] FunctionName2=...
                                ...
```

IFU

- There is a separate entry for the connected IFU in the PurityChrom.ini-file:

```
[KnauerIFU21]
Enabled=          [ 0/1 ]    OFF = 0, ON = 1
IP=               [ IP ]     IP-address
Port=             [ Port ]   port number of the COMport
DataRate=1       [ Value ]   datarate of the monitor
Channels=4       [ Value ]   number of channels
```

- If the IFU2.1 is connected via USB: the USB connection will be interpreted as COMport. Check your Windows Device Manager to identify the used COM port. To open the Device Manager (using Windows XP / 7) go to the "Control Panel" and open the "Device Manager". Please hide the IP address by using an inverted comma ('IP=)
- If you have an IFU 2.1LAN: please enter an IP-address
- Data rate: 1 - 10 Hz (4 channels), up to 50 Hz (channel 1 exclusively)
- For data acquisition using the IFU2.1 you have to choose a minimum Slide Width of 500 ms in PurityChrom (Time Table Editor, Function: Start Chromatogram)

Autosampler

- There is a separate entry for the connected Autosampler in the PurityChrom.ini-file:

[Autosampler]

TempPrecision=3	[°C]	Precision of set temperature
MaximumSamples=100	[Value]	Maximum sample capacity of the autosampler
VolumeDimension=µl	[µl]	Dimension of the injection volume. Please don't change this entry.
AS3000=0	[0/1]	The autosampler is a Labomatic AS3000.
VarioPrep=0	[0/1]	The autosampler is a SCPA VarioPrep.
Alias=0	[0/1]	The autosampler is a Spark Alias.
Knauer3950=0	[0/1]	The autosampler is a KNAUER 3950 or AS 6.1L.
Wash=0	[0/1]	1: washing cycle begins when starting the software 0: washing cycle after starting the software is turned off
TubingVolume=15	[µl]	Standard tubing volume (volume between needle and valve)
FlushVolume=30	[µl]	Standard flush volume (2x tubing volume)
PrepMode	[0/1]	Prep mode OFF = 0, Prep mode ON = 1 Knauer Autosampler with 10 ml Loop and 2,5 ml syringe volume
Transportliquid=	[0/1]	0 = Needle wash 1 = Transport wash
Transportvolume=	[1-9]	Number of syringe loads for washing

- The communication protocol for the KNAUER 3950/AS 6.1L has to be converted from UDP to TCP/IP. If necessary please request for SOP.

Direct Control

- To use the direct control function during the run to change the flowrate and the gradient composition change the PurityChrom.ini file as described here:

```
[Pumps]
Parameterchange=1
```

Tailing Factor instead of Asymmetry

- If the tailing factor should be calculated instead of the asymmetry, please change the PurityChrom.ini-file as described here:

```
[IntegrationPresets]

TailingFactor=1
```

Deactivate automatic Integration

- Please change the PurityChrom.ini-file as described here to deactivate the automatic integration:

```
[IntegrationPresets]

IntegrateInhibit=1,2,3.... (your data channels)
```

Using an external pressure signal

- Please change the PurityChrom.ini-file as described here to use an external pressure channel:

```
[Pumps]
PressureChannel=0 or 1-8 (0: no external pressure channel
                        1-8: number of your external pressure channel)
```

Demo chromatogram

- Starting PurityChrom in demo version, there is a possibility to run demo method
Choose the result file here:

```
[DemoMode]
Chromatogram=C: \PurityChrom\Data\ResultFileExample_0001.rfp
```

Tandem switching of two valves

- If you want to link the switching of two valves please change the PurityChrom.ini-file as described here:

```
[Tandem valve]
```

```
Index=... (number of tandem valve in the setup of PurityChrom)
SecondPort=...(Com-Port-Number of second valve) OR
SecondWinsockPort =...(Winsock of second valve)
```

You must not configure the second valve in the setup of PurityChrom.

13. Start the software. If the connection has been successful green ticks will appear behind the port number and if the connection was not successful a red cross will appear behind the port number.

14. Configuration of PurityChrom

- The menu option SETUP  in the main window will open the program settings window:

[Communication]

Please choose the correct Ports, baud rates and drivers for your devices according to the PurityChrom manual.

Note: Do not change the number of addresses in the Addr. Row!

Choose drivers “Multcom Interface” for fraction collector LABOCOL Vario 4000.

Valves:

The valves has to be configured additionally

Device	Address	Positions	Port	Type
Foxy R1/R2	1	2	Driver 5	Standard Valve
LABOCOL vario 4000	1	2	WinsockPort	Standard Valve
RID 2.1L	1	2	Driver 3	Standard Valve
P 6.1L HPG – SSV A	1	2	Driver 1	Standard Valve
P 6.1L HPG – SSV B	2	2	Driver 1	Standard Valve
Injection valve	1	2	WinsockPort	Knauer injection valve
Multiposition valve	1	Xx	WinsockPort	Knauer multiposition valve
Column selection valve	1	11	Com Port	Vici Valve

- Using trigger signal from the airsensor or a manual injection valve you can choose under “Control Inputs” to stop all, to start a run (Time Control Start) or to pause a run (Time Control Hold/Continue) depending on the signal. Using a manual injection valve connected to the pump via a trigger cable you have to choose “Event Box Input 1”. Using an airsensor connected via gameport adapter you have to choose “Gameport Input 1, 2, 3 or4)



[Presets]

Choose the right maximum pressure for the major and minor pump system.
Choose the right maximum flow rate for the major and minor pump system.

[Limiter]

The fraction limiter is a feature which calculates the volume of your fraction vial dependent on the flow rate. In the limiter setup the fraction limiter can be configured.

[Annotation]

The automatic annotations in the chromatograms are configured in the annotation setup

[Dead Time / Volume]

A considerable dead volume can occur in the tubing and valves between the detector cell and the waste/fraction switching valve, which results in a time delay when fractionating, especially at low flow rates. In the Dead Time / Dead Volume setup, you can define the dead volume or dead time of your system

[Description]

You can give your own names to the event box inputs and outputs, valves and the auxiliary output of the pump interface in the descriptions setup. This makes it easier to program time control files. It also makes the visualisations clearer, since the inputs, outputs and valves are can be listed by function.

Tip: Use user defined names in for your connected valves and their positions.

Injection Valve:	Position 1:	Load
	Position 2:	Inject
Valve of the Fraction Collector:	Position 1:	Waste
	Position 2:	Fraction
Multiposition Valve for Fractionation:	Position 1:	Waste
	Position 2:	Fraction 1...
Solvent Selection Valve A/B (HPG)	Position 1:	A1/B1
	Position 2:	A2/B2
Column Selection Valve (please set to ID 1, Baudrate 9600) This counts for all VICI valves	Position1-11	Bypass Column 1-5 Rev Column 1-5

Configuration of fraction valves OR Valves of fraction collector

[Limiter]

a) Limiter activation

Activate **Controlled Valve**, select the fraction valve or the valve of the fraction collector and choose **“Not waste”**

The fractionation should start if the fraction valve or the valve of your fraction collector changes the position from “Waste” to “Not waste”. Therefore, choose your fraction collector valve in “Controlled Valve”.

b) Limiter Output

Choose **Controlled Collector (Next Vial)**

[Annotation]

c) Waste Annotation Device

Choose the fraction valve or the valve of the fraction collector and **Waste**

d) Fraction Annotation Device

Choose the fraction valve or the valve of the fraction collector and **Not Waste**

e) Step Annotation Device

Choose **Controlled Collector Step**

[Dead Time / Volume]

f) Controlled Collector AND Controlled Valves

Enter here the delay volume (volume from detector cell to fraction collector)

Note: if no fraction valve or fraction collector is used, please deactivate Controlled Valve in the Limiter activation section

Configuration of P 6.1L HPG

Main Window configuration using P6.1L (high pressure gradient version)

- Using the P6.1L remove the buttons for C and D from the tool bar. Therefore, double-click on an empty space on the toolbar. The “Customize Toolbar” will open, where you can remove these two buttons. To hide a button, deselect the checkbox under it. The settings will be saved when you exit the program.
- Configuration of the solvent selection valve of P6.1L (high pressure gradient version)

[Communication]

a) Choose two standard valves:

The first valve can switch between A1 (position 1) and A2 position 2. Here you have to program following applies:

Addr.: 1, Pos.: 2, Port: Driver 1, Type: Standard Valve

The second valve can switch between B1 (position 1) and B2 position 2. Here you have to program following applies:

Addr.: 2, Pos.: 2, Port: Driver 1, Type: Standard Valve

Configuration DAD 2.1L/DAD 6,1L

Channels are configured in Time Control File Editor in section DAD and are saved to the method.

The wavelength symbol cannot be used to set the wavelength of DAD channels. Also the spectrum can be saved.

Information	Checklist	Variables	Options	Printout	DAD/MS	Functions	Threshold	Sampling
Data Selection								
	ADC	DAD	MID				Slicewidth ..:	100 ms
Channel 1 :1	<input type="radio"/>	<input checked="" type="radio"/>	<input type="radio"/>	210	-	220 nm	DAD	
Channel 2 :2	<input type="radio"/>	<input checked="" type="radio"/>	<input type="radio"/>	220	-	230 nm	Bandwidth ..:	1 nm
Channel 3 :3	<input type="radio"/>	<input checked="" type="radio"/>	<input type="radio"/>	230	-	240 nm	<input type="checkbox"/>	Save Spectrum
Channel 4 :4	<input type="radio"/>	<input checked="" type="radio"/>	<input type="radio"/>	240	-	250 nm	MID	
Channel 5 :5	<input type="radio"/>	<input checked="" type="radio"/>	<input type="radio"/>	250	-	260 nm	Mode	XIC
Channel 6 :6	<input type="radio"/>	<input checked="" type="radio"/>	<input type="radio"/>	260	-	270 nm	Attenuation ...:	25
Channel 7 :7	<input type="radio"/>	<input checked="" type="radio"/>	<input type="radio"/>	270	-	280 nm	Makeup Flow :	1000 µl
Channel 8 :8	<input type="radio"/>	<input checked="" type="radio"/>	<input type="radio"/>	280	-	290 nm	Range :	50.0 - 800.0
							<input type="checkbox"/>	Save Spectrum

Configuration of RID 2.1L

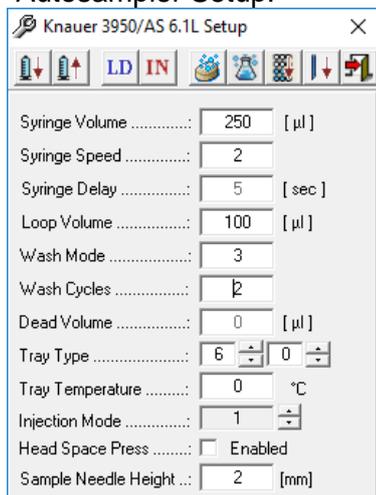
The flush valve needs to be configured separately:

Addr.: 1, Pos.: 2, Port: Driver 3, Type: Standard Valve

Data output is in nRIU. For µRIU please change the factor in the channel setting to 0,001.

Configuration of KNAUER 3950/AS 6.1L

In the menu option “File” in the main window of PurityChrom you will find the Autosampler Setup:



Syringe Volume	The volume of the autosampler syringe.
Syringe Speed	1: slow, 2: middle, 3: fast
Syringe Delay	Leave the syringe delay at 5 sec.
Loop Volume	The volume of your sample loop.
Wash Mode	0: washing cycle is turned off 2: between vials 3: between injection
Wash Cycles	How many wash cycles (1 – 3 times).
Dead Volume	Not important for KNAUER 3950/AS 6.1L.
Tray Type	0: Wellplate 96 low 1: Wellplate 96 high, 2: Wellplate 384 low 3: 48 vials 4: 12 vials 5: 108 vials (second tray don't need to be selected) 6: 84+3 vials (second tray don't need to be selected) 7: 30 vials (second tray don't need to be selected)
Tray Temperature	0°C: off Sample tray cooling for KNAUER 3950/AS 6.1L with cooling option: 4 – 40 °C
Injection Mode	0: None 1: Partial Loop Fill – max inject volume: 50 % of sample loop 2: Flushed Loop – full loop 3: Microliter Pickup – max inject volume: 50 % of sample loop
Head Space Press	Activate the HEAD SPACE PRESSURE option to support the sample transport to the sample loop by clicking.
Sample Needle Height	Select a distance between 2 and 6 mm

Methods with an autosampler injection are started about the autosampler control file or a sequence. Please read the manual for more information.

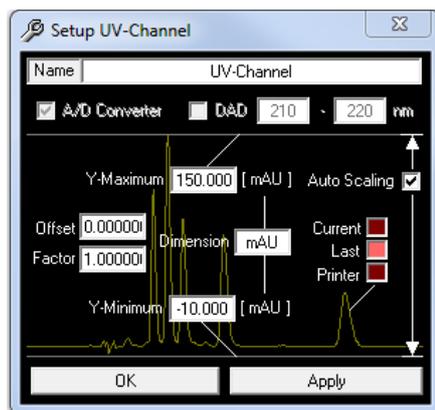
Stacked injection is not supported with KNAUER 3950/AS 6.1L.

Configuration of used data channels

Order of data channels:

1. UV
2. CM or RID
3. FLD or IFU channels
4. DAD

The menu option „Chromatogram“  in the main window opens the „Analysis window“. Using the icon „tools“  the selected channel can be configured (for example the name and the dimension). Read the manual for more information.



15. If the installation and configuration is finished, close the software. Copy the license file (PurityChrom.lic) on the CD (in folder: *License*) to C:\PurityChrom and insert the USB license dongle into a free USB port of the computer. The license will be recognized and can be used. Do not remove the dongle while PurityChrom is running.

16. KNAUER is not responsible for lost dongles. Losing the dongle means losing the license for software.

17. Restart your computer and the software PurityChrom once a week.

Attachment: IP addresses

- Use following IP addresses (subnet mask: 255.255.255.0, gateway: 192.168.1.1)

Device	IP-Address
Computer	192.168.1.100
Major Pumpe 1 (not in ASM 2.1L)	192.168.1.101
Major Pumpe 2 (binary HPG, not in ASM 2.1L)	192.168.1.102
Major Pumpe 3 (ternary HPG, not in ASM 2.1L)	192.168.1.103
Major Pumpe 4 (quaternary HPG, not in ASM 2.1L)	192.168.1.104
Assistant 1 (In the tower from bottom to top)	192.168.1.105
Assistant 2 (In the tower from bottom to top)	192.168.1.106
Assistant 3 (In the tower from bottom to top)	192.168.1.107
Assistant 4 (In the tower from bottom to top)	192.168.1.108
Assistant 5 (In the tower from bottom to top)	192.168.1.109
Detector	192.168.1.110
Conductivity monitor/RID 2.1L	192.168.1.111
Fraction Collector	192.168.1.112
Autosampler	192.168.1.113
Minor Pump (not in ASM 2.1L)	192.168.1.114
Ventil 1 (not in ASM 2.1L)	192.168.1.115
Ventil 2 (not in ASM 2.1L)	192.168.1.116
Ventil 3 (not in ASM 2.1L)	192.168.1.117
Ventil 4 (not in ASM 2.1L)	192.168.1.118
Ventil 5 (not in ASM 2.1L)	192.168.1.119

Mark devices with stickers: „Fixed IP-Address: 192.168.1.100“