GE Healthcare

Cue card 11-0014-58 AB

Automated multi-step protein purification

System preparations

Buffer suggestions

Column preparations

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Results and Evaluation

ÄKTA 3D plus Kit

System preparations

This cue card describes general system preparations

To ensure the best results, use high purity water and chemicals. It is also recommended to filter the solvents through a 0.45 µm filter.

Available protocols

Table 1. Steps in available protocols.

Protocol	Included steps
A	Affinity (AC) step
B	Affinity (AC) gradient
C	Affinity (AC) – Gel filtration (GF)
D	Affinity (AC) – Desalting (DS)
E	Affinity (AC) – Desalting (DS) – Ion Exchange (IEX)

Column positions

Table 2. Column positions for protocols A–E.

Column position	Protocol A	B	C	D	E
2	AC(1)	AC(1)	AC(1)	AC(1)	AC(1)
3	AC(2)	AC(2)	AC(2)	AC(2)	AC(2)
4	AC(3)	AC(3)	AC(3)	AC(3)	AC(3)
5	AC(4)	AC(4)	AC(4)	AC(4)	AC(4)
6	AC(5)	AC(5)	AC(5)	AC(5)	–
7	AC(6)	–	AC(6)	AC(6)	IEX
8	–	–	GF	DS	DS

System Pump

Fill buffer inlet tubings

- Prepare required buffers according to the chosen purification protocol. Add extra volume for system and column preparation.
- Place the inlet tubings in the buffers according to Table 3.
- If empty, purge the buffer inlet tubings manually according to P-900 User manual.

Table 3. Buffer positions for protocols A-E.

Buffer inlet	Protocol A	B	C	D	E
A11	AC binding	AC binding	AC binding	AC binding	AC binding
A12	–	–	GF	DS	IEX binding*
B1	AC elution	AC elution	AC elution	AC elution	AC elution
B2	–	–	–	–	IEX elution

* The IEX binding buffer is also used for desalting.

Wash system pump

- Open **System Control** and select **Manual:Pump.**
- Mark the instruction **Pump Wash Explorer** and select appropriate pump inlets (e.g. A11, B1, B2). Each pump wash requires approximately 50 ml buffer.
- Press **Execute** to start the wash.
- Press **End** when the wash is completed.

Note: *If more than one InletA1 needs to be washed (e.g. A11 and A12) the pump wash must be repeated.*

Sample Pump

Presence of air bubbles in the pump and/or sample flow path will affect the flow rate. Air must therefore be removed according to A or B below before starting the run.

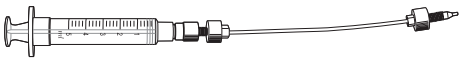
If the sample pump is empty and/or large amounts of the tubing is filled with air, proceed as in A below.

To remove small amounts of air, proceed as in B on next page.

A) Remove large amounts of air using a purge tubing

Purpose: To remove large amounts of air in the sample flow path by using a purge tubing and syringe.

- Put all the sample inlet tubings into affinity binding buffer.
- Set the sample valve V5 to any of the chosen sample inlet ports.
- Disconnect the connector fitted to injection valve V1, port 4.



- Connect the purge tubing to the same port.
- Make sure the injection valve V1 is set to **LOAD**.
- *Draw buffer with the syringe until liquid enters the syringe.
- *Switch the sample valve V5 to the next sample inlet tubing to be filled.
- Repeat the 2 steps above (marked with *) for the remaining sample inlet tubings.
- Disconnect the purge tubing.
- Connect the original connector to the port.

B) Remove small amounts of air from the sample tubing

Purpose: To remove small amounts of air in the sample flow path by running the sample pump manually.

- Put all the sample inlet tubings into affinity binding buffer.
- Open **System Control** and select **Manual:Flowpath**.
- Choose the instruction **SampleValve**, select the appropriate position and press **Execute**.
- Select **Pump** and choose **SampleFlow**.
- Enter an appropriate flow rate (e.g. 1 ml/min) and press **Execute**.
- When the selected inlet is filled with solution, change sample valve position and fill all sample inlets to be used the same way.
- When all sample inlets have been filled, set sample valve position to **S8** and run the sample pump at flow rate **40** ml/min for 1 min to make sure that no air remains in the sample flow path.

Change loop in Loop_1 Valve (V8)

By default, the 5 ml loop is connected to Loop_1 Valve (V8). If running AC-DS or AC-GF with large columns and large protein amounts, it is possible to increase the yield by using a 10 ml loop instead.

- Remove the 5 ml loop from the Loop_1 Valve (V8) and replace it with a 10 ml loop.

  Note: *If the loop is empty, fill it with buffer before the purification. Fill it manually with a syringe or use the template "System and Loop wash".*

- Create a method and check **Show details** in the Run Setup dialog.
- Change volumes for the variables displayed in the table below.

Table 4. Variables to change value of, if using a 10 ml loop instead of the default 5 ml loop in Loop_1 Valve (V8). Change values for all protocols that are used.

Block/Variable	Default[1] (ml)	New[2] (ml)
Wash_Loop1_Che/**Wash_Volume_Loop[1]_Che[3]**	25	50
or		
Wash_Loop1_GST/**Wash_Volume_Loop[1]_GST[3]**	25	50
Hold_Until_Peak_Less_Than/**Max_CollectionVolume**	3.5	6.5
Inject_Collected_peak/**Loop_1_Volume**	4	7
Delay_inject_selected_peak/**InjectionDelay**	5	10

[1] The default values are for 5 ml HiTrap columns.
[2] The new values are examples that are useable if using a HiPrep Desalting or a HiLoad Superdex 26/60 in the second step.
[3] Depending on the type of column used.

Buffer suggestions and column preparations

This cue card describes how to prepare different columns before running a protocol

To ensure the best results, use high purity water and chemicals. It is also recommended to filter the solvents through a 0.45 µm filter.

Buffer suggestions

AC buffer suggestions for His-tagged proteins

If performing...	suggested buffer
binding using HisTrap	50 mM Tris-HCl pH 7.5, 0.5 M NaCl, 20-40 mM imidazole1)
binding using HiTrap Chelating	50 mM Tris-HCl pH 7.5, 0.5 M NaCl, 5–40 mM imidazole1)
elution	50 mM Tris-HCl pH 7.5, 0.5 M NaCl, 500 mM imidazole

[1] The imidazole concentration is protein dependent.

AC buffer suggestions for GST-tagged proteins

If performing...	suggested buffer
binding using GSTrap HP or FF	50 mM Tris-HCl pH 7.5, 150 mM NaCl, 1 mM DTT
elution	50 mM Tris-HCl, 0.15 M NaCl, 1 mM DTT, 10 mM reduced gluthathione, pH 8

DS buffer suggestions

If preparing for...	suggested buffer
AIEX	50 mM Tris-HCl pH 8.0
CIEX	20 mM MES pH 6.0
protein storage	include e.g. 10% glycerol in a suitable buffer (e.g. 50 mM Tris-HCl, pH 7.5, 150 mM NaCl)

IEX buffer suggestions

If for example...	suggested buffer (depends on the pI of the protein)
binding to AIEX	50 mM Tris-HCl pH 8.0
binding to CIEX	20 mM MES pH 6.0
eluting from AIEX	50 mM Tris-HCl pH 8.0, 1 M NaCl
eluting from CIEX	20 mM MES pH 6.0, 1 M NaCl

GF buffer suggestions

If preparing for...	suggested buffer
further studies	a suitable buffer, e.g. 50 mM Tris-HCl pH 7.5, 150 mM NaCl
protein storage	include e.g. 10% glycerol in a suitable buffer (e.g. 50 mM Tris-HCl pH 7.5, 150 mM NaCl)

Column preparations

Affinity Columns

Metal ion charging

Purpose: To charge new or stripped HiTrap Chelating HP or HisTrap columns with metal ions (e.g. Ni^{2+}, Co^{2+} or Cu^{2+}). Several columns can be prepared automatically.

Create the method

- Open **Method Editor** and select **Template** under **File:New**.
- Select the **Metal Ion Charging 1ml** or **5ml** template and press **OK**.
- Open the Scouting page. Use **Add** to create the number of runs equal to the number of columns that should be charged.

  Note: *Even if only one column will be used, a scouting run still has to be added.*
- Define column positions for each run.

  Note: *Default Column Position is set to Position1Bypass, which means no column.*
- Save the method.

Prepare the system

- Prepare required solutions (see Figure 1).
- Connect the chelating columns at defined column positions.
- Place the inlet tubings in the solutions according to Figure 1.
- Fill the inlet tubings S1, S2, and S8 and purge the sample pump according to the instructions in the "System preparations" cue card.

Start the method.

The method takes approximately 15 minutes/column to run.

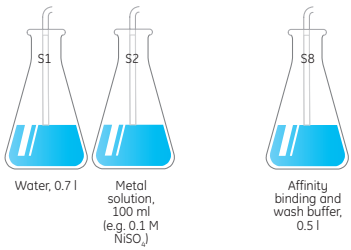


Fig 1. Solutions required to run the "Metal Ion Charging" template.

Affinity blank run

Purpose: Prior to a first time usage of an affinity column (GSTrap FF, GSTrap HP or a newly metal charged HiTrap Chelating HP or HisTrap) it is recommended to run a blank run. This ensures a well-conditioned and equilibrated column ready for chromatography.

Create the method

- Open **Method Editor** and select **Template** under **File:New**.
- Select the **Affinity Blank Run 1ml** or **5ml** template and press **OK**.
- Open the Scouting page. Use **Add** to create the number of runs equal to the number of columns that should be equilibrated. Select to wash inlet A11 and B1 in the first run.

  Note: *Even if only one column will be used, a scouting run still has to be added.*

- Define column positions for each run.

  Note: *Default Column Position is set to Position1Bypass, which means no column.*

- Save the method.

Prepare the system

- Prepare required buffers, see Figure 2.
- Place the inlet tubings in the buffers according to Figure 2.
- If empty, purge the inlet tubings (A11 and B1) according to the system pump instructions in the "System preparations" cue card.
- Connect the affinity columns at defined column positions.

Start the method.

The method takes approximately 10 minutes/column to run.

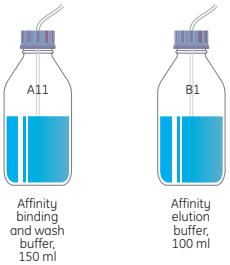


Fig 2. Buffers required to run the "Affinity Blank Run" template.

Ion exchange, Desalting and Gel filtration columns

Equilibrate columns

Purpose: To remove EtOH and equilibrate columns with buffer.

Prepare the system

- Prepare sufficient volume of the required buffer, see Table 1.
- Place the inlet tubing (e.g. A12) in the equilibration buffer and fill the inlet according to the system pump instructions given in the "System preparation" cue card.
- Connect the columns.

Manual run

Equilibration of columns is performed manually from **System Control**.

Note: *After each selection below always press* ***Execute****.*

- Select **Alarm_Pressure** in **Manual:Alarms&Mon**, and key in HighAlarm, see Table 1.
- Select **BufferValveA1** (A12) in **Manual:Flowpath**.
- Select appropriate **ColumnPosition** in **Manual:Flowpath**.
- Select **Flow** in **Manual:Pump**, key in appropriate flow, see Table 1.
- Select **End_timer** in **Manual:Other** and choose **Acc volume**, key in appropriate Timeout volume, see Table 1.
- When the first column is ready, repeat for the next column to be equilibrated.

  Note: *To make automated methods, see UNICORN™ User Manual.*

Table 1. Recommended HighAlarms, Flow rates and Equilibration volumes for different columns, when running ÄKTA 3D plus Kit including a 0.2 MPa flow restrictor.

Column	High Alarm (MPa)	Flow rate removing EtOH (ml/min)	Flow rate equilibration (ml/min)	Timeout Equilibration volume (ml)
HiLoad 16/60	0.5	0.5	1.0	360
HiLoad 26/60	0.5	1.3	2.5	954
HiPrep 26/10	0.35	7.5	15.0	159
HiTrap, 1ml	0.5	0.5	1.0	5
HiTrap, 5ml	0.5	2.5	5.0	25
RESOURCE™, 1ml	1.5	2.0	4.0	5
RESOURCE, 6ml	1.5	3.0	6.0	30
Mono Q™ 5/50 GL	4.0	1.0	2.0	5
Mono S™ 5/50 GL	4.0	1.0	2.0	5

Protocol A: Affinity – step elution

This cue card describes how to run this specific purification protocol

Before starting a purification protocol make sure to prepare system and columns according to the instructions on the system preparations, buffer suggestions and column preparations cue cards.

1 Prepare your UNICORN method

- Open the method wizard and select purification protocol **A) Affinity (Step)**.
- Mark the number of samples **1 to 6** to be purified.
- Press **Next**.
- Choose columns.
- Indicate your running condition; **room temperature** or **cold room**.
- Press **Finish** to obtain the purification method.
- It is possible to change default values in the method, if needed. Check the **Show details** box. For more details, see ÄKTA 3D plus Kit User Manual.
- Save your method.

2 Prepare your system before a run

- Prepare buffers required for this protein purification protocol according to the "Buffer suggestions and column preparations" cue card.
- Place buffer inlets as shown in Figure 1 and make sure your system is prepared as described in the "System preparations" cue card.
- Connect selected columns as shown in Figure 2 and prepare them according to the "Buffer suggestions and column preparations" cue card.
- Load the fraction collector with four 96-well microplates (2 ml). You will also need to load the fraction collector with 50 ml fraction tubes to collect the second wash of the affinity column. For more details, see ÄKTA 3D plus Kit User Manual.

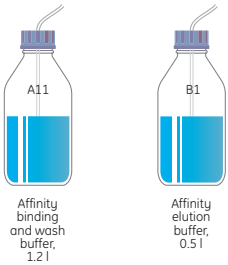


Fig 1. Buffer inlets and maximum volumes needed, see ÄKTA 3D plus Kit User Manual for details.

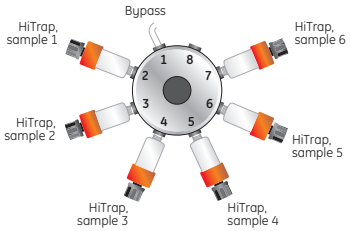


Fig 2. Column connections at column valves V2 and V3.

Prepare and load samples

- Prepare your samples and clarify them using centrifugation and/or filtration through a 0.45 µm filter.
- Prepare the sample pump according to the "System preparations" cue card. Gently transfer each sample inlet tubing into each sample according to Figure 3. Make sure no air enters the tubing.

Fig 3. Sample placement at the sample valve V5.

4 Start the method

- Start your prepared method.

In the start protocol:

- Key in sample ID for the proteins on the variable page. Each chromatogram will be named after the sample ID.

5 Evaluate results

- How to view the results in an easy way, and how to calculate protein concentrations, is described in ÄKTA 3D plus Kit User Manual and on the "Results and evaluation" cue card.

Protocol B: Affinity – gradient elution

This cue card describes how to run this specific purification protocol

Before starting a purification protocol make sure to prepare system and columns according to the instructions on the system preparations, buffer suggestions and column preparations cue cards.

1 Prepare your UNICORN method

- Open the method wizard and select purification protocol **B) Affinity (Gradient)**.
- Mark the number of samples **1 to 5** to be purified.
- Press **Next**.
- Choose columns.
- Indicate your running condition; **room temperature** or **cold room**.
- Press **Finish** to obtain the purification method.
- It is possible to change default values in the method, if needed. Check the **Show details** box. For more details, see ÄKTA 3D plus Kit User Manual.
- Save your method.

2 Prepare your system before a run

- Prepare buffers required for this protein purification protocol according to the "Buffer suggestions and column preparations" cue card.
- Place buffer inlets as shown in Figure 1 and make sure your system is prepared as described in the "System preparations" cue card.
- Connect selected columns as shown in Figure 2 and prepare them according to the "Buffer suggestions and column preparations" cue card.
- Load the fraction collector with four 96-well microplates (2 ml). You will also need to load the fraction collector with 50 ml fraction tubes to collect the second wash of the affinity column. For more details, see ÄKTA 3D plus Kit User Manual.

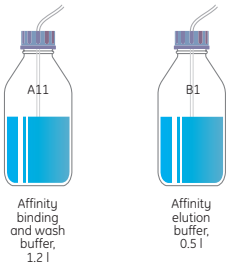


Fig 1. Buffer inlets and maximum volumes needed, see ÄKTA 3D plus Kit User Manual for details.

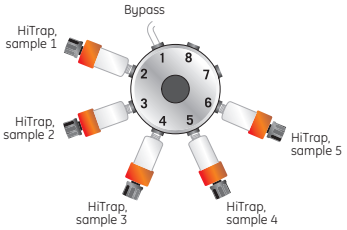


Fig 2. Column connections at column valves V2 and V3.

Prepare and load samples

- Prepare your samples and clarify them using centrifugation and/or filtration through a 0.45 µm filter.
- Prepare the sample pump according to the "System preparations" cue card. Gently transfer each sample inlet tubing into each sample according to Figure 3. Make sure no air enters the tubing.

Fig 3. Sample placement at the sample valve V5.

4 Start the method

- Start your prepared method.

In the start protocol:

- Key in sample ID for the proteins on the variable page. Each chromatogram will be named after the sample ID.
- Start your automated multi-step purification.

5 Evaluate results

- How to view the results in an easy way, and how to calculate protein concentrations, is described in ÄKTA 3D plus Kit User Manual and on the "Results and evaluation" cue card.

Protocol C: Affinity – Gel filtration

This cue card describes how to run this specific purification protocol

Before starting a purification protocol make sure to prepare system and columns according to the instructions on the system preparations, buffer suggestions and column preparations cue cards.

1 Prepare your UNICORN method

- Open the method wizard and select purification protocol **C) Affinity (Step) – Gel Filtration**.
- Mark the number of samples **1 to 6** to be purified.
- Press **Next**.
- Choose columns. See Table 1 for recommendations.
- Indicate your running condition; **room temperature** or **cold room**.
- Press **Finish** to obtain the purification method.
- It is possible to change default values in the method, if needed. Check the **Show details** box. For more details, see ÄKTA 3D plus Kit User Manual.
- Save your method.

Table 1. Recommended column combinations.

Goal	First column	Second column	Frac-950
Up to 10 mg protein	HiTrap[1], 1 ml	HiLoad 16/60 Superdex™ 75 prep grade or HiLoad 16/60 Superdex 200 prep grade	Rack C with micro titre plates and 50 ml tubes
Up to 50 mg protein	HiTrap[1], 5 ml	HiLoad 26/60 Superdex™ 75 prep grade or HiLoad 26/60 Superdex 200 prep grade	Rack A with 10 and 50 ml tubes

[1] Either of HiTrap Chelating HP, HisTrap, Any HiTrap, GSTrap FF or GSTrap HP can be used.

2 Prepare your system before a run

- Prepare buffers required for this protein purification protocol according to the "Buffer suggestions and column preparations" cue card.
- Place buffer inlets as shown in Figure 1 and make sure your system is prepared as described in the "System preparations" cue card.
- Connect selected columns as shown in Figure 2 and prepare them according to the "Buffer suggestions and column preparations" cue card.
- Fill the fraction collector with four 96-well plates (2 ml) or 120 tubes (10 ml) depending on your choice of gel filtration column (see Table 1). You will also need to fill the fraction collector with 50 ml fraction tubes to collect the second wash of the affinity column (one tube per sample is needed).

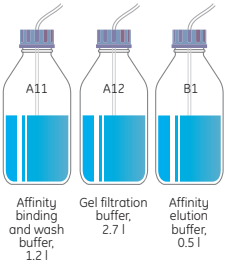


Fig 1. Buffer inlets and maximum volumes needed, see ÄKTA 3D plus Kit User Manual for details.

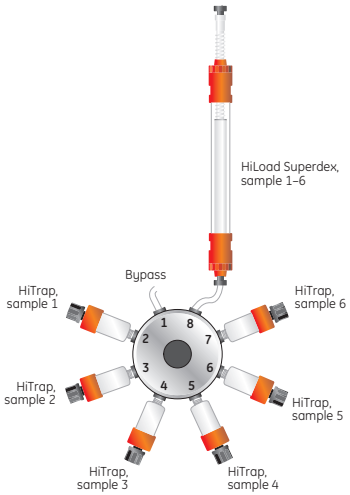


Fig 2. Column connections at column valves V2 and V3.

3 Prepare and load samples

- Prepare your samples and clarify them using centrifugation and/or filtration through a 0.45 µm filter.
- Prepare the sample pump according to the "System preparations" cue card. Gently transfer each sample inlet tubing into each sample according to Figure 3. Make sure no air enters the tubing.

Fig 3. Sample placement at the sample valve V5.

4 Start the method

- Start your prepared method.

In the start protocol:

- Key in sample ID for the proteins on the variable page. Each chromatogram will be named after the sample ID.
- Start your automated multi-step purification.

5 Evaluate results

- How to view the results in an easy way, and how to calculate protein concentrations, is described in ÄKTA 3D plus Kit User Manual and on the "Results and evaluation" cue card.

Protocol D: Affinity – Desalting

This cue card describes how to run this specific purification protocol

Before starting a purification protocol make sure to prepare system and columns according to the instructions on the system preparations, buffer suggestions and column preparations cue cards.

1 Prepare your UNICORN method

- Open the method wizard and select purification protocol **D) Affinity (Step) – Desalting**.
- Mark the number of samples **1 to 6** to be purified.
- Press **Next**.
- Choose columns. See Table 1 for recommendations.
- Indicate your running condition; **room temperature** or **cold room**.
- Press **Finish** to obtain the purification method.
- It is possible to change default values in the method, if needed. Check the **Show details** box. For more details, see ÄKTA 3D plus Kit User Manual.
- Save your method.

Table 1. Recommended column combinations.

Goal	First column	Second column	Frac-950
Up to 10 mg protein	HiTrap[1], 1 ml	2 × HiTrap DS or HiPrep DS	Rack C with micro titre plates and 50 ml tubes
Up to 50 mg protein	HiTrap[1], 5 ml	2 × HiTrap DS or HiPrep DS	Rack C with micro titre plates and 50 ml tubes

[1] Either of HiTrap Chelating HP, HisTrap, Any HiTrap, GSTrap FF or GSTrap HP can be used.

2 Prepare your system before a run

- Prepare buffers required for this protein purification protocol according to the "Buffer suggestions and column preparations" cue card.
- Place buffer inlets as shown in Figure 1 and make sure your system is prepared as described in the "System preparations" cue card.
- Connect selected columns as shown in Figure 2 and prepare them according to the "Buffer suggestions and column preparations" cue card.
- Load the fraction collector with four 96-well micro-plates (2 ml). You will also need to load the fraction collector with 50 ml fraction tubes to collect the second wash of the affinity column. For more details, see ÄKTA 3D plus Kit User Manual.

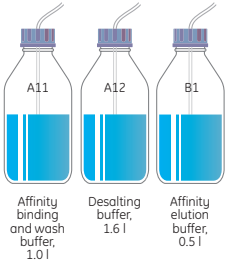


Fig 1. Buffer inlets and maximum volumes needed, see ÄKTA 3D plus Kit User Manual for details.

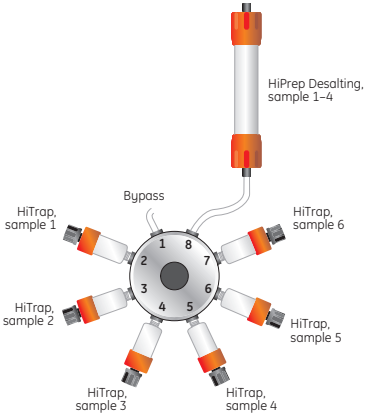


Fig 2. Column connections at column valves V2 and V3.

3 Prepare and load samples

- Prepare your samples and clarify them using centrifugation and/or filtration through a 0.45 µm filter.
- Prepare the sample pump according to the "System preparations" cue card. Gently transfer each sample inlet tubing into each sample according to Figure 3. Make sure no air enters the tubing.

Fig 3. Sample placement at the sample valve V5.

4 Start the method

- Start your prepared method.

In the start protocol:

- Key in sample ID for the proteins on the variable page. Each chromatogram will be named after the sample ID.
- Start your automated multi-step purification.

5 Evaluate results

- How to view the results in an easy way, and how to calculate protein concentrations, is described in ÄKTA 3D plus Kit User Manual and on the "Results and evaluation" cue card.

Protocol E: Affinity – Desalting– Ion Exchange

This cue card describes how to run this specific purification protocol

Before starting a purification protocol make sure to prepare system and columns according to the instructions on the system preparations, buffer suggestions and column preparation cue cards.

1 Prepare your UNICORN method

- Open the method wizard and select purification protocol **E) Affinity (step) – desalting – IEX**.
- Mark the number of samples **1 to 4** to be purified.
- Press **Next**.
- Choose columns. See Table 1 for recommendations.
- Indicate your running condition; **room temperature** or **cold room**.
- Press **Finish** to obtain the purification method.
- It is possible to change default values in the method, if needed. Check the **Show details** box. For more details, see ÄKTA 3D plus Kit User Manual.
- Save your method.

Table 1. Recommended column combinations.

Goal	First column	Second column	Third column	Frac-950
Up to 10 mg protein	HiTrap[1], 1 ml	2 × HiTrap Desalting or HiPrep Desalting	1, 5 or 6 ml IEX[2]	Rack C with micro titre plates and 50 ml tubes
Up to 50 mg protein	HiTrap[1], 5 ml	HiPrep Desalting	5 or 6 ml IEX[2]	Rack C with micro titre plates and 50 ml tubes

[1] Either of HiTrap Chelating HP, HisTrap, Any HiTrap, GSTrap FF or GSTrap HP can be used.

[2] AIEX for proteins with low pI, CIEX for proteins with high pI.

2 Prepare your system before a run

- Prepare buffers required for this protein purification protocol according to the "Buffer suggestions and column preparations" cue card.
- Place buffer inlets as shown in Figure 1 and make sure your system is prepared as described in the "System preparations" cue card.
- Connect selected columns as in Figure 2 and prepare them according to the "Buffer suggestions and column preparations" cue card.
- Load the fraction collector with four 96-well microplates (2 ml). You will also need to load the fraction collector with 50 ml fraction tubes to collect the second wash of the affinity column and the IEX flowthrough. (Two tubes per sample are needed). For more details, see ÄKTA 3D plus Kit User Manual.

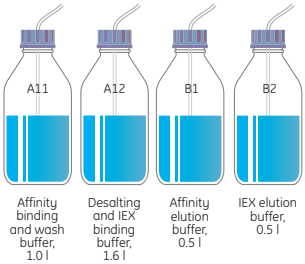


Fig 1. Buffer inlets and maximum volumes needed, see ÄKTA 3D plus Kit User Manual for details.

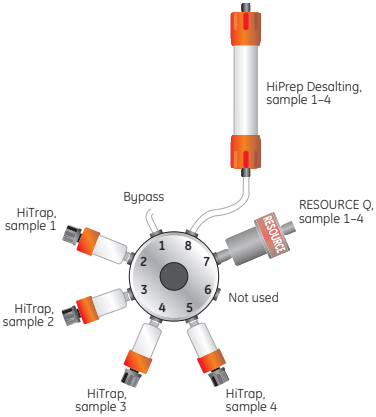


Fig 2. Column connections at column valves V2 and V3.

3 Prepare and load samples

- Prepare your samples and clarify them using centrifugation and/or filtration through a 0.45 µm filter.
- Prepare the sample pump according to the "System preparations" cue card. Gently transfer each sample inlet tubing into each sample according to Figure 3. Make sure no air enters the tubing.

Fig 3. Sample placement at the sample valve V5.

4 Start the method

- Start your prepared method.

In the start protocol:

- Key in sample ID for the proteins on the variable page. Each chromatogram will be named after the sample ID.
- Start your automated multi-step purification.

5 Evaluate results

- How to view the results in an easy way, and how to calculate protein concentrations, is described in ÄKTA 3D plus Kit User Manual and on the "Results and evaluation" cue card.

System maintenance and column cleaning

This cue card describes how to clean your system and columns after performed protein purification

To ensure the best results, use high purity water and chemicals. It is also recommended to filter buffers through a 0.45 µm filter.

System and Loop Wash

Purpose: To wash all used tubings within the system, including the loops.

Create the method

- Open Method Editor and select **Template** under **File:New**.
- Select the **System and Loop Wash** template and press **OK**.
- Select up to two buffer inlets to be washed at the same time as the system (e.g. A11 and B1).

  Note: *If more than one* ***InletA1*** *needs washing (e.g. A11 and A12) we recommend to clean these inlets according to the "System Pump" instructions in the "System preparations" cue card before performing the System Wash.*
  Sample inlet tubings must be washed separately according to the "Sample Pump" instructions in the "System preparations" cue card.
- Save the method.

Prepare the system

- Prepare a sufficient volume (0.4 l) of the wash solution.
- Place the inlet tubings in the wash solution.

Start the method.

The method takes approximately 10 minutes to run.

Column CIP (cleaning-in-place)

Purpose: To clean contaminated columns. For column cleaning procedures and column storage instructions, please refer to the instructions supplied with each column or to our homepage: **www.gelifesciences.com/chromatography**

Create the method

- Open Method Editor and select **Template** under **File:New**.
- Select the **Column CIP** template and press **OK**.
- Open the Scouting page. Use **Add** to create the number of runs equal to the number of columns that should be cleaned.

  Note: *Even if only one column will be used, a scouting run still has to be added.*
- Define **Column volume, Pressure limit, Regulation pressure, Flow rate** and **Column position** for each run. See table 1 for recommendations.
- Select the buffer inlets, flow rates and volumes for all solutions that will be used. Up to 10 steps per column can be performed.
- Save the method.

Prepare the system

- Prepare a sufficient volume of each required solution.
- Connect the columns at defined column positions.
- Place the inlet tubings in appropriate solutions.
- Fill the inlet tubings according to "System Pump" instructions in the "System preparations" cue card.

Start the method.

Table 1. CIP values to enter on the scouting page if using FR-902 flow restrictor.

Column	Column volume (ml)	Pressure limit (MPa)	Regulation pressure[1] (MPa)	Flow rate RT[2] (ml/min)	Flow rate CR3 (ml/min)
HiTrap 1 ml	0.96	0.5	0.45	1.0	0.8
HiTrap 5 ml	5.0	0.5	0.45	5.0	4.0
HiPrep DS	53	0.35	0.3	10	8.0
RESOURCE 1 ml	0.97	1.5	1.3	4.0	3.2
RESOURCE 6 ml	6.0	1.5	1.3	6.0	4.8
Mono Q 5/50 GL	0.98	4.0	3.6	2.0	1.6
Mono S 5/50 GL	0.98	4.0	3.6	2.0	1.6
HiLoad 16/60	121	0.5	0.45	1.0	0.8
HiLoad 26/60	319	0.5	0.45	2.5	2.0

[1] Target pressure used by the pressure/flow regulation. Should be lower than the pressure limit.
[2] RT=room temperature
[3] CR= cold room

Metal Ion Stripping (chelating columns)

Purpose: To remove metal ions before regenerating the HiTrap HP and HisTrap columns. To recharge the column, follow the instructions given on the "Buffer suggestions and column preparations" cue card.

Note: *Always remove metal ions before or right after storing the HiTrap Chelating and HisTrap columns in EtOH.*

Create the method

- Open Method Editor and select **Template** under **File:New**.
- Select the **Metal Ion Stripping 1ml** or **5ml** template and press **OK**.
- Open the Scouting page. Use **Add** to create the number of runs equal to the number of columns that should be stripped.

  Note: *Even if only one column will be used, a scouting run still has to be added.*
- Define **Column Position** for each run.
- Save the method.

Prepare the system

- Prepare required solutions, see Figure 1.
- Connect the columns at defined column positions.
- Place the inlet tubings in the solutions according to Figure 1.
- Fill the inlet tubings S1, S3 and S8, and purge the sample pump according to instructions in the "System preparations" cue card.

Start the method.

The method takes approximately 20 minutes/column to run.

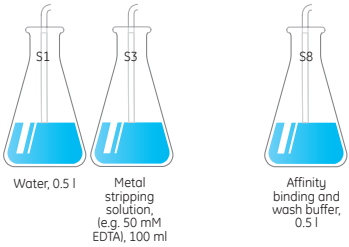


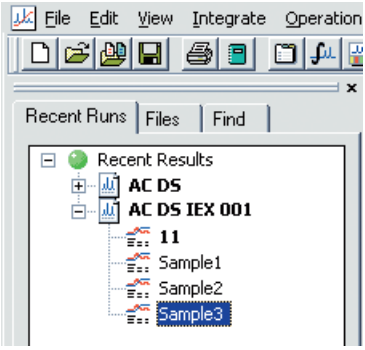
Fig 1. Solutions required for the "Metal Ion Stripping" template.

Results and evaluation

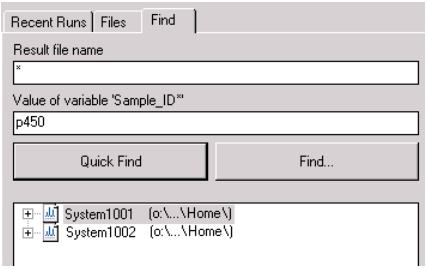
This cue card describes how to open results and determine protein concentration and amount

1 Find and open the result files

- Use the **Recent Runs** or the **Find** tab, in the Evaluation module to locate the result file.
- Click ⊞ to expand the list for the result file.
  A result file consists of:
  – Sample loading chromatogram (11 below)
  – Purification chromatograms for each sample
- Double-click a sample ID to open that specific sample chromatogram.
- Double-click a result file to open all included chromatograms.
  (The first chromatogram shows the loading of all samples onto the affinity columns. Each one of the remaining chromatograms shows the purification of one individual sample.)



Note: *To locate a specific sample, click the* ***Find*** *tab, enter sample ID and click* ***Quick Find****.*



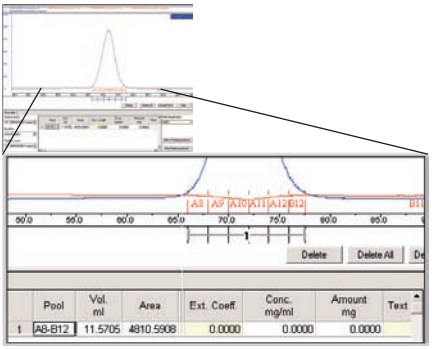
2 Select curves to display

- Right-click on the chromatogram and select **Properties**.
- In the curve selection table select curves to be displayed (commonly; **1**, **4**, **6**, **11** and **22**). (If the **Clear** function is used, also remember to scroll down and select curve **22**.)
- Press **OK** to confirm your selections.

Note: *If you have entered the sample ID on the variable page, the sample ID will be shown in the chromatogram heading.*

3 Pool fractions and adjust pooling

- If required, maximize the sample window ⧉.
- Zoom in on the relevant fractions.
- Choose **Operations:Pool** to pool the fractions.
  The pooled fractions are listed in a table below the chromatogram and the pooled peaks are numbered sequentially in the chromatogram.
  Only adjacent fractions will be pooled. The fraction numbers for each pool are listed in the table as a range in retention order, e.g. A6–A7 etc.
- If necessary, adjust pooling.

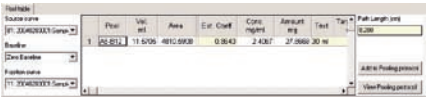


4 Determine protein concentration and amount

- Enter the real cell lengt of the UV cell in the **Path Lenght (cm)** field.

  Note: *If the real cell lenght has been set direcly in Monitor UV-900, enter the nominal cell lenght (0.2 cm) instead.*

- Enter the extinction coefficient manually by marking a pool and then typing the value in the extinction coefficient field.

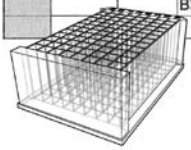


The concentration is given in mg/ml, or M, and amount is given in mg, or mmole, depending on the coefficient used.

- Click the **Add to Pooling Protocol** button to add the adjusted pools to the Pooling protocol.
- Repeat the procedure for other chromatograms from the same, or other, result files.
- Click the **View Pooling Protocol** button.

Pooling protocol

System	Result	Pool	Vol.	Conc.	Text
SYS_1					
	AC DS001				
		A2-A5			
			7.5620	2.9275	
		A7-B12			
			12.7663	3.7228	
		B10-B5			
			11.2592	3.5886	
		B3-C2			
			9.5732	0.8292	



5 Print or save the Pooling protocol

The Pooling protocol can be used as a help when making the physical pooling of the purified samples from the microplate.

- *To print the Pooling protocol:*
  Click the **Print** button to print the protocol on the default printer.
- *To save the Pooling protocol as a file:*
  Click **Export** and save the protocol in one of the following formats: text (.txt), Excel (.xls), HTML (.htm).

Ordering information

Product	Pack Size	Code No.
ÄKTA 3D plus Kit*, with software, valve (INV-907), air sensor (915-N), flow restrictor (FR-902) tubings, sample loops, fittings, instruction manual and cue cards	1	11-0014-53
ÄKTAexplorer 100	please contact your local sales representative of GE Healthcare	
Fraction collector Frac-950, complete with Rack A, 18 and 30 mm tubes	1	18-6083-00
Rack C, complete with bowl for 96-well microtitre plates** and 30 mm tubes	1	18-6083-13
Supported Columns:		
Affinity chromatography		
HiTrap Chelating HP	5 × 1 ml	17-0408-01
HiTrap Chelating HP	1 × 5 ml	17-0409-01
HisTrap HP	5 × 1 ml	17-5247-01
HisTrap HP	5 × 5 ml	17-5248-02
GSTrap FF	5 × 1 ml	17-5130-01
GSTrap FF	2 × 1 ml	17-5130-02
GSTrap FF	1 × 5 ml	17-5131-01
GSTrap HP	5 × 1 ml	17-5281-01
GSTrap HP	5 × 5 ml	17-5282-02
Gel filtration		
HiLoad 16/60 Superdex 75 prep grade	1 × 120 ml	17-1068-01
HiLoad 16/60 Superdex 200 prep grade	1 × 120 ml	17-1069-01
HiLoad 26/60 Superdex 75 prep grade	1 × 318 ml	17-1070-01
HiLoad 26/60 Superdex 200 prep grade	1 × 318 ml	17-1071-01
Buffer exchange		
HiPrep 26/10 Desalting	1 × 53 ml	17-5087-01
HiTrap Desalting	5 × 5 ml	17-1408-01
Ion exchange chromatography		
RESOURCE Q	1 × 1 ml	17-1177-01
RESOURCE Q	1 × 6 ml	17-1179-01
RESOURCE S	1 × 1 ml	17-1178-01
RESOURCE S	1 × 6 ml	17-1180-01
HiTrap Q HP	5 × 1 ml	17-1153-01
HiTrap Q HP	5 × 5 ml	17-1154-01
HiTrap SP HP	5 × 1 ml	17-1151-01
HiTrap SP HP	5 × 5 ml	17-1152-01
HiTrap Q FF	5 × 1 ml	17-5053-01
HiTrap Q FF	5 × 5 ml	17-5156-01
HiTrap SP FF	5 × 1 ml	17-5054-01
HiTrap SP FF	5 × 5 ml	17-5157-01
HiTrap DEAE FF	5 × 1 ml	17-5055-01
HiTrap DEAE FF	5 × 5 ml	17-5154-01
HiTrap CM FF	5 × 1 ml	17-5056-01
HiTrap CM FF	5 × 5 ml	17-5155-01
HiTrap ANX FF (high sub)	5 × 1 ml	17-5162-01
HiTrap ANX FF (high sub)	5 × 5 ml	17-5163-01
HiTrap Q XL	5 × 1 ml	17-5158-01
HiTrap Q XL	5 × 5 ml	17-5159-01
HiTrap SP XL	5 × 1 ml	17-5160-01
HiTrap SP XL	5 × 5 ml	17-5161-01
Mono Q 5/50 GL	1 × 1 ml	17-5166-01
Mono S 5/50 GL	1 × 1 ml	17-5168-01

Related products and literature:

ÄKTA 3D plus Kit User Manual	1	11-0014-57
Test Kit 280 nm (2 mm cell)	1	18-1129-63
ÄKTA 3D plus Kit Miniposter	1	11-0025-34
HiTrap Column Guide	1	18-1129-81
ÄKTAexplorer chromatography systems Data File	1	18-1124-09
Fraction collector Frac-950 Data File	1	18-1153-57
Affinity Chromatography Handbook	1	18-1022-29
Gel Filtration Handbook	1	18-1022-18
Ion Exchange Chromatography Handbook	1	18-1114-21
Recombinant Protein Handbook	1	18-1142-75
GST Gene Fusion System Handbook	1	18-1157-58
Purifying Challenging Proteins Handbook	1	28-9095-31

* ÄKTAexplorer100 (with Pump P-960 and UNICORN 5.01 or higher) and Fraction collector Frac-950 (with Rack A and Rack C; Rack A is the standard rack supplied with Fraction collector Frac-950) are needed to use ÄKTA 3D plus Kit. ÄKTAexplorer 100 upgrades can be ordered from your local LabcrewTM representative of GE Healthcare. A representative of GE Healthcare is required to install ÄKTA 3D plus Kit.

** GE Healthcare recommends Greiner, PP-Masterblock, 2 ml, 96 well: 780270

Trouble shooting and useful hints can be found in the User Manual: 11-0014-57 AA.

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