GE Healthcare

ÄKTAprime plus

Cue Cards

Contents

ÄKTA

System Preparation

1 Starting the system

a) Press the button on the back of the system.

b) Wait until the system self-test has been completed (30-40 seconds). **Templates** is displayed when the system is ready to use.

2 Starting the computer

a) Start the computer.

b) Start PrimeView™.

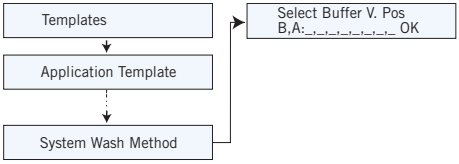
c) Check the communication between system and computer.

Tip! If communication has been established, the text **Controlled By: prime** should be displayed at the lower right corner of the screen. If not, check the connections.

3 Purging the system flow path

a) *If the flow path is filled with storage solution (e.g. ethanol 20%), remove it as follows:*

- Place the three brown waste tubings in waste.
- Set all inlet tubings (B, A1, ...) that will be used in water (see the appropriate cue card).
- Select the application template **System Wash Method**, select the inlets to be washed. Press **OK**.

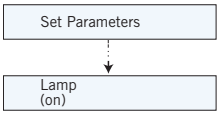


b) *If there are large amounts of air in the tubing:*
Fill the tubing using Purge kit P-950. See *ÄKTAprime™ plus User Manual* for detailed instructions.

4 Preparing the monitors

a) Check the UV lamp filter position and the lamp position.

b) Check that the UV lamp is on.



Note: Wait at least 15 minutes before using the system after the lamp has been turned on to avoid drifting UV base line.

c) *If using pH:*
Calibrate the pH. The pH must be calibrated every day. See *ÄKTAprime plus User Manual* for instructions.

5 Checking the flow rate

a) Set the injection valve to position WASTE.

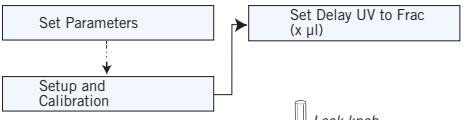
b) Start the flow (1-50 ml/min).

c) Use a graduated glass cylinder to collect at the WASTE outlet (port 5). Collect during at least one minute.

Note: Inaccurate flow rate may be due to air in the pump. If this is the case, flush the pump with buffer and try again. If the problem persists, flush the system with ehtanol or methanol followed by water.

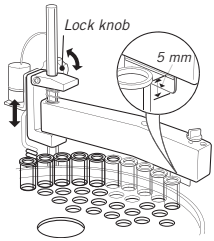
6 Preparing the fraction collector

a) Check that the delay volume is correct. The delay volume is equal to the volume between the UV flow cell and the fraction collector (default 380 µl).

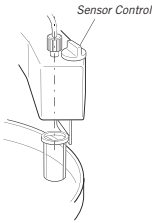


b) Fill the fraction collector with tubes*.

c) Adjust the height of the delivery arm so that the horizontal mark on the tube sensor is at the same level as the top of the collection tubes.



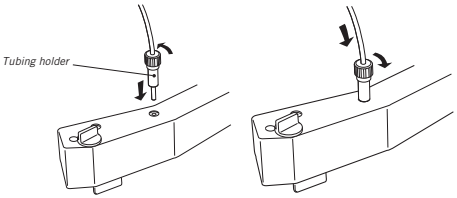
d) Check that the sensor is in the correct position for the tube size. The eluent tubing should be positioned above the center of the collection tube. Use the red sensor control to position the tube holder.



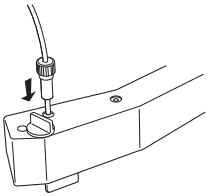
* See each cue card for information on the number of tubes to be used.

e) Place the tubing holder over the length guide (small hole) in the delivery arm, push the tubing down to the bottom of the guide and tighten the nut. This ensures that the correct length of the tubing is exposed.

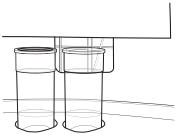
Note: Make sure that the end of the tubing is cut leaving a straight edge. For more information, see *ÄKTAprime plus User Manual* .



f) Re-install the tubing holder into the delivery arm.

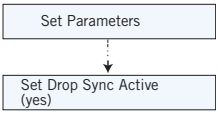


g) Rotate the rack by hand until the rear half of the tube sensor rests against tube 1.



h) Press **feed tube** on the front panel. The bowl moves to the correct position to collect the first fraction in tube 1.

i) Make sure that drop synchronization is activated.



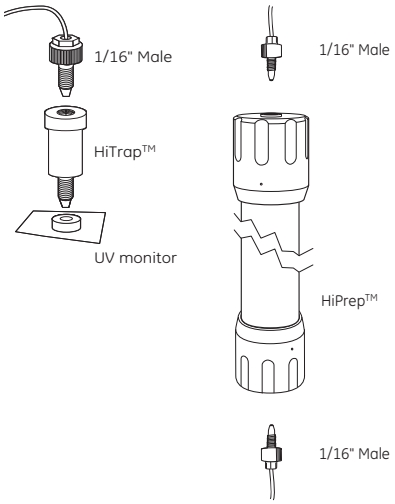
Note: Drop synchronization can only be used at flow rates ≤ 3 ml/min.

7 Cleaning the sample loop and connecting the column

a) Mount a sample loop between port 2 and port 6 that is large enough to hold your sample.

b) *Clean the loop and the injector fill port:*
Inject (with a syringe) 5 times the loop volume of water or binding buffer through the injection fill port.

c) Connect the column between port 1 on the injection valve (7-port valve) and the UV flow cell.



8 Filling the buffer inlet tubing

Prepare the buffers. Make sure that correct inlet tubing is put into correct buffer.

- *If using an application template:*
  It is not necessary to perform the wash procedure because this is included in the method.
- *If using a method template:*
  Pre-fill all inlet tubings with buffer. Select the application template **System Wash Method**, select the inlets to be washed and press **OK**.

The system is now prepared. Start the method.

9 Storage of the system

a) *Clean the loop and the injector fill port:*
- Inject (with a syringe) 5 times the loop volume of water through the injection fill port.
- Repeat this step with 20% ethanol.

b) *Flush the system flow paths:*
- Put all used inlet tubings in water.
- Select the application template **System Wash Method,** select the inlets to be washed and press **OK**.
- For long-term storage of the system, repeat step b) with 20% ethanol.

c) Shut down the system.

Buffer exchange on HiTrap Desalting

1 Preparing the buffers

- Use high purity water and chemicals.
- Filter all buffers through a 0.45 µm filter before use.

Buffer (port A1):
20 mM sodium phosphate, 0.15 M NaCl, pH 7.0*

Prepare at least 500 ml eluent.

* When performing buffer exchange use the appropriate buffer.

2 Preparing the sample

Pass the sample through a 0.45 µm filter.

The maximum recommended sample volume is 1.5 ml.

3 Preparing the system

a) Place the inlet tubing from port A1 (8-port valve) and port B (2-port valve) in the buffer.

b) Place the three brown waste tubings in waste.

c) Connect the column between port 1 on the injection valve (7-port valve) and the UV flow cell (see Ordering information on next page for suitable columns).

d) Fill the fraction collector rack with 18 mm tubes (minimum 20) and position the white plate on the fractionation arm against the first tube.

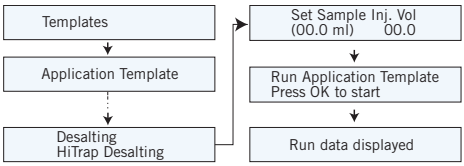
e) Connect a sample loop large enough for your sample between port 2 and 6 on the injection valve. Use a syringe to manually fill the loop.

Note: If the same sample is applied repeatedly, a Superloop™ can be used. For information of how to use it see the instructions for the Superloop.

4 Selecting Application Template and starting the method

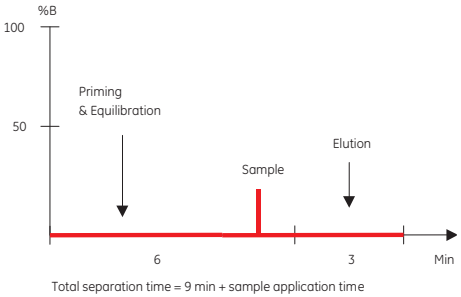
a) Check the communication to PrimeView. At the lower right corner of the screen the text **Controlled By: prime** should be displayed.

b) Use the arrow and OK buttons to move in the menu tree until you find **Desalting HiTrap Desalting**.



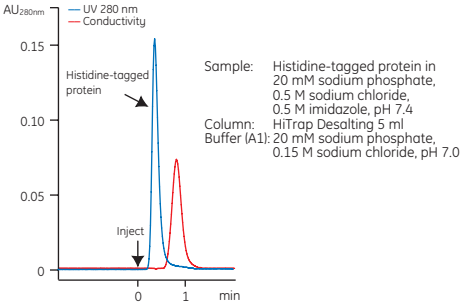
c) Enter the sample volume and press **OK** to start the template.

Theoretical gradient in **Desalting HiTrap Desalting** *Application Template.*



Total separation time = 9 min + sample application time

5 Typical result



Troubleshooting

High backpressure:

- *Column clogged* – Clean the column according to instructions. Make sure the sample has been centrifuged and/or filtered through a 0.45 µm filter.
- *System clogged* – Replace the column with a piece of tubing. Check pressure. If backpressure > 0.3 MPa, clean system according to manual.

Eluted sample still contaminated:

- Check that the sample load limit is not exceeded.

Ordering information

Product	Quantity	Code No.
HiTrap Desalting	5 × 5 ml	17-1408-01
	100 × 5 ml*	11-0003-29
Superloop 10 ml	1	18-1113-83

* Pack size available by special order

Buffer exchange on HiPrep 26/10 Desalting

1 Preparing the buffers

- Use high purity water and chemicals.
- Filter all buffers through a 0.45 µm filter before use.

Buffer (port A1):
20 mM sodium phosphate, 0.15 M NaCl, pH 7.0*

Prepare at least 500 ml eluent.

* When performing buffer exchange use the appropriate buffer.

2 Preparing the sample

Pass the sample through a 0.45 µm filter.

The maximum recommended sample volume is 15 ml.

3 Preparing the system

a) Place the inlet tubing from port A1 (8-port valve) and port B (2-port valve) in the buffer.

b) Place the three brown waste tubings in waste.

c) Connect the column between port 1 on the injection valve (7-port valve) and the UV flow cell (see Ordering information on next page for suitable columns).

d) Fill the fraction collector rack with 18 mm tubes (minimum 25) and position the white plate on the fractionation arm against the first tube.

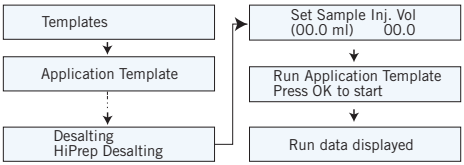
e) Connect a sample loop large enough for your sample between port 2 and 6 on the injection valve. Use a syringe to manually fill the loop.

Note: If a Superloop is needed, additional information is supplied in the instructions for Superloop.

4 Selecting Application Template and starting the method

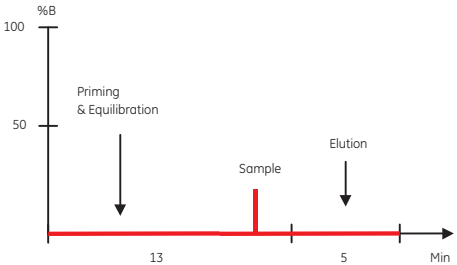
a) Check the communication to PrimeView. At the lower right corner of the screen the text **Controlled By: prime** should be displayed.

b) Use the arrow and OK buttons to move in the menu tree until you find **Desalting HiPrep Desalting**.



c) Enter the sample volume and press **OK** to start the template.

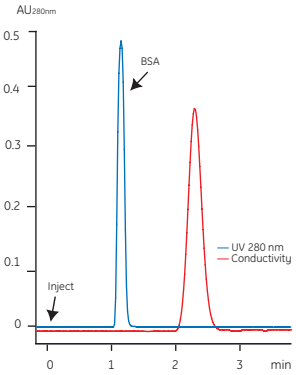
Theoretical gradient in **Desalting HiPrep Desalting** *Application Template.*



Total separation time = 18 min + sample application time

5 Typical result

Sample: BSA and sodium chloride
Column: HiPrep 26/10 Desalting, 53 ml
Buffer (A1): 20 mM sodium phosphate, 0.15 M NaCl, pH 7.0



Troubleshooting

High backpressure:

- *Column clogged* – Clean the column according to instructions. Make sure the sample has been centrifuged and/or filtered through a 0.45 µm filter.
- *System clogged* – Replace the column with a piece of tubing. Check pressure. If backpressure > 0.3 MPa, clean system according to manual.

Eluted sample still contaminated:

- Check that the sample load limit is not exceeded.

Ordering information

Product	Quantity	Code No.
HiPrep 26/10 Desalting	1 (53 ml)	17-5087-01
	4 (53 ml)	17-5087-02
Superloop 10 ml	1	18-1113-83
Superloop 50 ml	1	18-1113-84

Histidine-tagged protein purification, step elution

1 Preparing the buffers

- Use high purity water and chemicals.
- Filter all buffers through a 0.45 µm filter before use.

Binding buffer (port A1):
20 mM sodium phosphate, 0.5 M NaCl, 20 mM imidazole, pH 7.4 *

Elution buffer (port B):
20 mM sodium phosphate, 0.5 M NaCl, 0.5 M imidazole, pH 7.4

Prepare at least 500 ml of each eluent.

* *Alternative binding buffers:*
5–40 mM imidazole can be included in the binding buffer to reduce unspecific binding of non-histidine-tagged proteins. The concentration of imidazole is protein dependent and if the protein of interest elutes or does not bind at a certain imidazole concentration, then reduce the concentration.

2 Preparing the sample

a) Adjust the sample to composition of binding buffer by:
- diluting the sample in binding buffer *or*
- by buffer exchange using HiTrap Desalting or HiPrep 26/10 Desalting.

b) Pass the sample through a 0.45 µm filter.

Note: If HisTrap FF crude column is used no filtration nor clarification of the sample is needed.

3 Preparing the system

a) Place the inlet tubing from port A1 (8-port valve) in the binding buffer and the tubing from port B (2-port valve) in the elution buffer.

b) Place the three brown waste tubings in waste.

c) Connect the column between port 1 on the injection valve (7-port valve) and the UV flow cell (see Ordering information on next page for suitable columns).

d) Fill the fraction collector rack with 18 mm tubes** and position the white plate on the fractionation arm against the first tube.

** The number of tubes to insert in the fraction collector varies with the sample volume. Fill the fraction collector with 20 tubes + one tube/ml sample. For example, if the sample volume is 10 ml, fill the fraction collector with 20 + 10 = 30 tubes. However, note that the maximum capacity of the fraction collector is 95 tubes, limiting the sample volume to 75 ml.

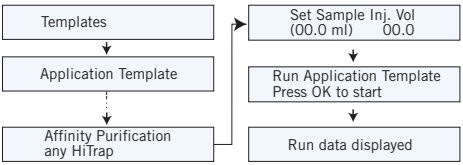
e) Connect a sample loop large enough for your sample between port 2 and 6 on the injection valve. Use a syringe to manually fill the loop.

Note: If a Superloop is needed, additional information is supplied in the instructions for Superloop.

4 Selecting Application Template and starting the method

a) Check the communication to PrimeView. At the lower right corner of the screen the text **Controlled By: prime** should be displayed.

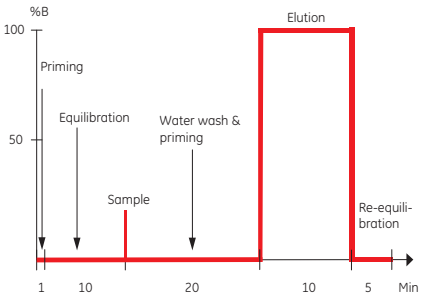
b) Use the arrow and OK buttons to move in the menu tree until you find **Affinity Purification any HiTrap**.



c) Enter the sample volume and press **OK** to start the template.

Note: If a 5 ml column is preferred, see cue card on p.36.

Theoretical gradient in **Affinity Purification any HiTrap** *Application Template.*

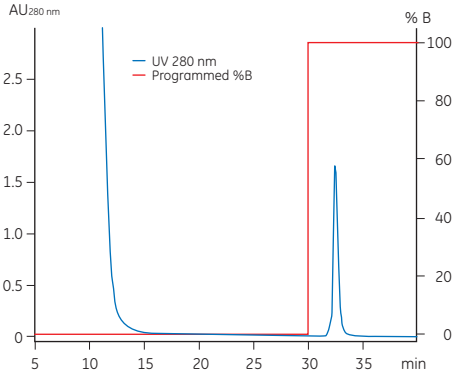


Total separation time = 47 min + sample application time

5 Typical result

Sample: Clarified homogenate of *E. coli* expressing histidine-tagged protein
Column: HisTrap™ HP 1 ml

Binding buffer (port A1): 20 mM phosphate, 0.5 M NaCl, 20 mM imidazole, pH 7.4
Elution buffer (port B): 20 mM phosphate, 0.5 M NaCl, 0.5 M imidazole, pH 7.4



Troubleshooting

High backpressure:

- *Column clogged* – Clean the column according to instructions. Make sure the sample has been centrifuged and/or filtered through a 0.45 µm filter. For unclarified samples using HisTrap FF crude, make sure the sample has been lysed properly, e.g. using thorough sonication and DNase treatment.
- *System clogged* – Replace the column with a piece of tubing. Check pressure. If backpressure > 0.3 MPa, clean system according to manual.

No binding:

- Check that the correct column is used.
- Check that the inlet tubing from each buffer is connected to the correct inlet port.
- Check that the composition and pH of the buffers are correct.
- Check that the sample has been adjusted to binding buffer conditions.
- Check that your sample contains target protein.

No elution:

- Check that the inlet tubing from each buffer is connected to the correct inlet port.
- Check that the composition and pH of the buffers are correct.
- Use alternative elution conditions according to the column instructions.
- Check that your sample contains target protein.

Ordering information

Product	Quantity	Code No.
HisTrap HP	5 × 1 ml	17-5247-01
	100 × 1 ml*	17-5247-05
HisTrap FF	5 × 1 ml	17-5319-01
	100 × 1 ml*	17-5319-02
HisTrap FF crude	5 × 1 ml	11-0004-58
	100 × 1 ml*	11-0004-59
HiTrap Desalting	5 × 5 ml	17-1408-01
	100 × 5 ml*	11-0003-29
HiPrep 26/10 Desalting	1 (53 ml)	17-5087-01
	4 (53 ml)	17-5087-02
Superloop 10 ml	1	18-1113-83
Superloop 50 ml	1	18-1113-84
Superloop 150 ml	1	18-1023-85

* Pack size available by special order

Histidine-tagged protein purification, gradient elution

1 Preparing the buffers

- Use high purity water and chemicals.
- Filter all buffers through a 0.45 µm filter before use.

Binding buffer (port A1):
20 mM sodium phosphate, 0.5 M NaCl, 20 mM imidazole, pH 7.4 *

Elution buffer (port B):
20 mM sodium phosphate, 0.5 M NaCl, 0.5 M imidazole, pH 7.4

Prepare at least 500 ml of each eluent.

* *Alternative binding buffers:*
5–40 mM imidazole can be included in the binding buffer to reduce unspecific binding of non-histidine-tagged proteins. The concentration of imidazole is protein dependent and if the protein of interest elutes or does not bind at a certain imidazole concentration, then reduce the concentration

2 Preparing the sample

a) Adjust the sample to composition of binding buffer by:

- diluting the sample in binding buffer *or*
- by buffer exchange using HiTrap Desalting or HiPrep 26/10 Desalting.

b) Pass the sample through a 0.45 µm filter.

Note: If HisTrap FF crude column is used, nor filtration nor clarification of the sample is needed.

3 Preparing the system

a) Place the inlet tubing from port A1 (8-port valve) in the binding buffer and the tubing from port B (2-port valve) in the elution buffer.

b) Place the three brown waste tubings in waste.

c) Connect the column between port 1 on the injection valve (7-port valve) and the UV flow cell (see Ordering information on next page for suitable columns).

d) Fill the fraction collector rack with 18 mm tubes (minimum 40) and position the white plate on the fractionation arm against the first tube.

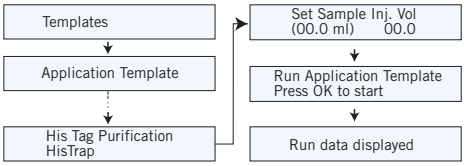
e) Connect a sample loop large enough for your sample between port 2 and 6 on the injection valve. Use a syringe to manually fill the loop.

Note: If a Superloop is needed, additional information is supplied in the instructions for Superloop.

4 Selecting Application Template and starting the method

a) Check the communication to PrimeView. At the lower right corner of the screen the text **Controlled By: prime** should be displayed.

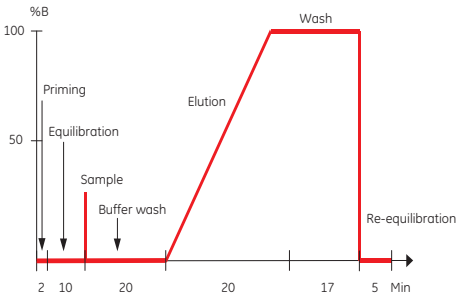
b) Use the arrow and OK buttons to move in the menu tree until you find **His Tag Purification HisTrap**.



c) Enter the sample volume and press **OK** to start the template.

Note: If a 5 ml column is preferred see cue card on p.36.

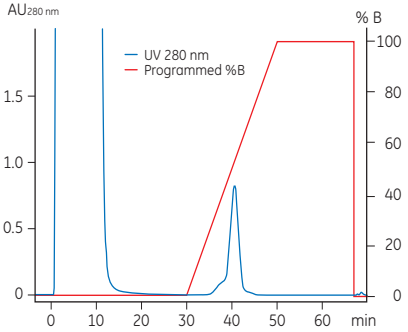
Theoretical gradient in **His Tag Purification HisTrap** *Application Template.*



Total separation time = 74 min + sample application time

5 Typical result

Sample: Clarified homogenate of *E. coli* expressing histidine-tagged protein
Column: HisTrap HP 1 ml
Binding buffer (port A1): 20 mM phosphate, 0.5 M NaCl, 20 mM imidazole, pH 7.4
Elution buffer (port B): 20 mM phosphate, 0.5 M NaCl, 0.5 M imidazole, pH 7.4



Troubleshooting

High backpressure:

- *Column clogged* – Clean the column according to instructions. Make sure the sample has been centrifuged and/or filtered through a 0.45 µm filter. For unclarified samples using HisTrap FF crude, make sure the sample has been lysed properly, e.g. using thorough sonication and DNase treatment.
- *System clogged* – Replace the column with a piece of tubing. Check pressure. If backpressure > 0.3 MPa, clean system according to manual.

No binding:

- Check that the correct column is used.
- Check that the inlet tubing from each buffer is connected to the correct inlet port.
- Check that the composition and pH of the buffers are correct.
- Check that the sample has been adjusted to binding buffer conditions.
- Check that your sample contains target protein.

No elution:

- Check that the inlet tubing from each buffer is connected to the correct inlet port.
- Check that the composition and pH of the buffers are correct.
- Use alternative elution conditions according to the column instructions.
- Check that your sample contains target protein.

Ordering information

Product	Quantity	Code No.
HisTrap HP	5 × 1 ml	17-5247-01
	100 × 1 ml*	17-5247-05
HisTrap FF	5 × 1 ml	17-5319-01
	100 × 1 ml*	17-5319-02
HisTrap FF crude	5 × 1 ml	11-0004-58
	100 × 1 ml*	11-0004-59
HiTrap Desalting	5 × 5 ml	17-1408-01
	100 × 5 ml*	11-0003-29
HiPrep 26/10 Desalting	1 (53 ml)	17-5087-01
	4 (53 ml)	17-5087-02
Superloop 10 ml	1	18-1113-83
Superloop 50 ml	1	18-1113-84
Superloop 150 ml	1	18-1023-85

* Pack size available by special order

IMAC purification - any metal

1 Preparing the buffers

- Use high purity water and chemicals.
- Filter all buffers through a 0.45 µm filter before use.

Binding buffer (port A1):
20 mM sodium phosphate, 0.5 M NaCl, 20 mM imidazole, pH 7.4 *

Wash eluent (port A2):
Distilled water

Metal-loading eluent (port A3):
100 mM metal salt solution (metal chloride or metal sulphate e.g. 100 mM $CuSO_4$, 100 mM $CoCl_2$ or 100 mM $ZnCl_2$ in distilled H_2O)

Elution buffer (port B):
20 mM sodium phosphate, 0.5 M NaCl, 0.5 M imidazole, pH 7.4

Prepare at least 500 ml of each eluent.

* *Alternative buffers:*
5–40 mM imidazole can be included in the binding buffer to reduce unspecific binding of non-histidine-tagged proteins. The concentration of imidazole is protein dependent and if the protein of interest elutes or does not bind at a certain imidazole concentration, then reduce the concentration.

2 Preparing the sample

a) Adjust the sample to composition of binding buffer by:
- diluting the sample in binding buffer *or*
- by buffer exchange using HiTrap Desalting or HiPrep 26/10 Desalting.

b) Pass the sample through a 0.45 µm filter.

3 Preparing the system

a) Place each inlet tubing from port A (8-port valve) in eluents as given above and the tubing from port B (2-port valve) in the elution buffer.

b) Place the three brown waste tubings in waste.

c) Connect the column between port 1 on the injection valve (7-port valve) and the UV flow cell (see Ordering information on next page for suitable columns).

d) Fill the fraction collector rack with 18 mm tubes (minimum 40) and position the white plate on the fractionation arm against the first tube.

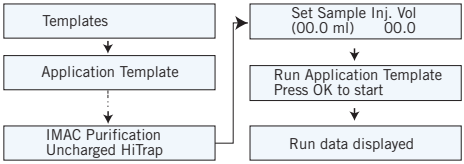
e) Connect a sample loop large enough for your sample between port 2 and 6 on the injection valve. Use a syringe to manually fill the loop.

Note: If a Superloop is needed, additional information is supplied in the instructions for Superloop.

4 Selecting Application Template and starting the method

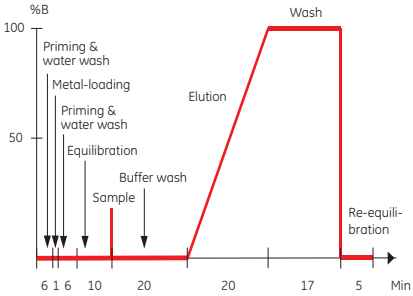
a) Check the communication to PrimeView. At the lower right corner of the screen the text **Controlled By: prime** should be displayed.

b) Use the arrow and OK buttons to move in the menu tree until you find **IMAC Purification Uncharged HiTrap**.



c) Enter the sample volume and press **OK** to start the template.
Note: If a 5 ml column is preferred, see cue card on p.36.

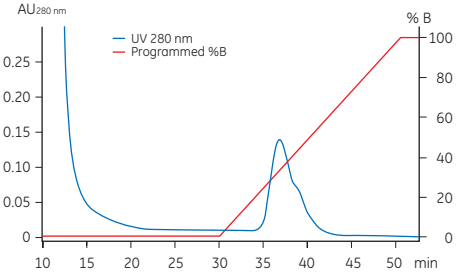
Theoretical gradient in **IMAC Purification Uncharged HiTrap** *Application Template.*



Total separation time = 87 min + sample application time

5 Typical result

Sample:	Clarified homogenate of *E. coli* expressing histidine-tagged protein
Column:	HiTrap Chelating HP 1 ml
Binding buffer (port A1):	20 mM phosphate, 0.5 M NaCl, 20 mM imidazole, pH 7.4
Wash eluent (port A2):	Distilled water
Metal-loading eluent (port A3):	100 mM $ZnCl_2$ in distilled water
Elution buffer (port B):	20 mM phosphate, 0.5 M NaCl, 0.5 M imidazole, pH 7.4



Troubleshooting

High backpressure:

- *Column clogged* – Clean the column according to instructions. Make sure the sample has been centrifuged and/or filtered through a 0.45 µm filter.
- *System clogged* – Replace the column with a piece of tubing. Check pressure. If backpressure > 0.3 MPa, clean system according to manual.

No binding:

- Check that the correct column is used.
- Check that the inlet tubing from each buffer is connected to the correct inlet port.
- Check that the composition and pH of the buffers are correct.
- Check that the sample has been adjusted to binding buffer conditions.
- Check that your sample contains target protein.

No elution:

- Check that the inlet tubing from each buffer is connected to the correct inlet port.
- Check that the composition and pH of the buffers are correct.
- Use alternative elution conditions according to the column instructions.
- Check that your sample contains target protein.

Ordering information

Product	Quantity	Code No.
HiTrap IMAC HP	5 × 1 ml	17-0920-03
HiTrap IMAC FF	5 × 1 ml	17-0921-02
HiTrap Chelating HP	5 × 1 ml	17-0408-01
HiTrap Desalting	5 × 5 ml	17-1408-01
	100 × 5 ml*	11-0003-29
HiPrep 26/10 Desalting	1 (53 ml)	17-5087-01
	4 (53 ml)	17-5087-02
Superloop 10 ml	1	18-1113-83
Superloop 50 ml	1	18-1113-84
Superloop 150 ml	1	18-1023-85

* Pack size available by special order

On-column refolding

1 Preparing the buffers

- Use high purity water and chemicals.
- Filter all buffers through a 0.45 µm filter before use.

Binding buffer (port A1):
6 M guanidine hydrochloride, 20 mM Tris-HCl, 0.5 M NaCl, 5 mM imidazole, 1 mM 2-mercaptoethanol, pH 8.0*

Solubilisation buffer (port A2):
6 M urea, 20 mM Tris-HCl, 0.5 M NaCl, 5 mM imidazole, 1 mM 2-mercaptoethanol, pH 8.0**

Elution buffer (port A3):
20 mM Tris-HCl, 0.5 M NaCl, 0.5 M imidazole, 1 mM 2-mercaptoethanol, pH 8.0

Refolding buffer (port B):
20 mM Tris-HCl, 0.5 M NaCl, 5 mM imidazole, 1 mM 2-mercaptoethanol, pH 8.0**

Prepare at least 500 ml of each eluent.

* *Alternative binding buffers:*
5–40 mM imidazole can be included in the binding buffer to reduce unspecific binding of non-histidine-tagged proteins. The concentration of imidazole is protein dependent and if the protein of interest elutes or does not bind at a certain imidazole concentration, then reduce the concentration.

** Include the same imidazole concentration as in used binding buffer.

2 Preparing the sample

a) Adjust the sample to composition of binding buffer by:
- diluting the sample in binding buffer *or*
- by buffer exchange using HiTrap Desalting or HiPrep 26/10 Desalting.

b) Pass the sample through a 0.45 µm filter.

3 Preparing the system

a) Place each inlet tubing from port A (8-port valve) in eluents as given above and the tubing from port B (2-port valve) in the elution buffer.

b) Place the three brown waste tubings in waste.

c) Connect the column between port 1 on the injection valve (7-port valve) and the UV flow cell (see Ordering information on next page for suitable columns).

d) Fill the fraction collector rack with 18 mm tubes (minimum 40) and position the white plate on the fractionation arm against the first tube.

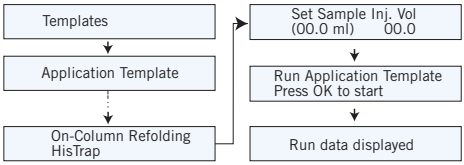
e) Connect a sample loop large enough for your sample between port 2 and 6 on the injection valve. Use a syringe to manually fill the loop.

Note: If a Superloop is needed, additional information is supplied in the instructions for Superloop.

4 Selecting Application Template and starting the method

a) Check the communication to PrimeView. At the lower right corner of the screen the text **Controlled By: prime** should be displayed.

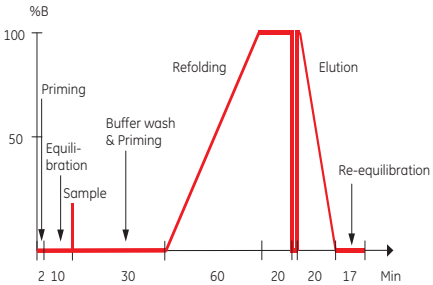
b) Use the arrow and OK buttons to move in the menu tree until you find **On-Column Refolding HisTrap**.



c) Enter the sample volume and press **OK** to start the template.

Note: If a 5 ml column is preferred see cue card on p.36.

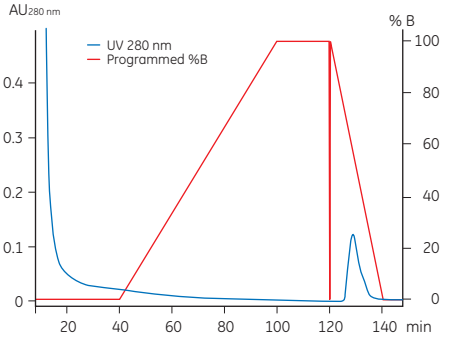
Theoretical gradient in **On-column Refolding HisTrap** *Application Template.*



Total separation time = 160 min + sample application time

5 Typical result

Sample:	Clarified homogenate of *E. coli* expressing histidine-tagged protein
Column:	HisTrap FF 1 ml
Binding buffer (A1):	6 M Guanidine hydrochloride, 20 mM Tris-HCl, 0.5 M NaCl, 5 mM Imidazole, 1mM 2-mercaptoethanol, pH 8.0
Solubilization buffer (port A2):	6 M Urea, 20 mM Tris-HCl, 0.5 M NaCl, 5 mM Imidazole, 1 mM 2-mercaptoethanol, pH 8.0
Elution buffer (port A3):	20 mM Tris-HCl, 0.5 M NaCl, 0.5 M Imidazole, 1 mM 2-mercaptoethanol, pH 8.0
Refolding buffer (port B):	20 mM Tris-HCl, 0.5 M NaCl, 5 mM Imidazole, 1 mM 2-mercaptoethanol, pH 8.0



Troubleshooting

High backpressure:

- *Column clogged* – Clean the column according to instructions. Make sure the sample has been centrifuged and/or filtered through a 0.45 µm filter.
- *System clogged* – Replace the column with a piece of tubing. Check pressure. If backpressure > 0.3 MPa, clean system according to manual.

No binding:

- Check that the correct column is used.
- Check that the inlet tubing from each buffer is connected to the correct inlet port.
- Check that the composition and pH of the buffers are correct.
- Check that the sample has been adjusted to binding buffer conditions.
- Check that your sample contains target protein.

No elution:

- Check that the inlet tubing from each buffer is connected to the correct inlet port.
- Check that the composition and pH of the buffers are correct.
- Use alternative elution conditions according to the column instructions.
- Check that your sample contains target protein.

Ordering information

Product	Quantity	Code No.
HisTrap HP	5 × 1 ml	17-5247-01
	100 × 1 ml*	17-5247-05
HisTrap FF	5 × 1 ml	17-5319-01
	100 × 1 ml*	17-5319-02
HiTrap Desalting	5 × 5 ml	17-1408-01
	100 × 5 ml*	11-0003-29
HiPrep 26/10 Desalting	1 (53 ml)	17-5087-01
	4 (53 ml)	17-5087-02
Superloop 10 ml	1	18-1113-83
Superloop 50 ml	1	18-1113-84
Superloop 150 ml	1	18-1023-85

* Pack size available by special order

GST-tagged protein purification

1 Preparing the buffers

- Use high purity water and chemicals.
- Filter all buffers through a 0.45 µm filter before use.

Binding buffer (port A1):
20 mM sodium phosphate, 0.15 M NaCl, pH 7.3
Elution buffer (port B):
50 mM Tris-HCl, 10 mM reduced glutathione, pH 8.0

Prepare at least 500 ml of each eluent.

2 Preparing the sample

a) Adjust the sample to composition of binding buffer by:
- diluting the sample in binding buffer *or*
- by buffer exchange using HiTrap Desalting or HiPrep 26/10 Desalting.

b) Pass the sample through a 0.45 µm filter.

3 Preparing the system

a) Place the inlet tubing from port A1 (8-port valve) in the binding buffer and the tubing from port B (2-port valve) in the elution buffer.

b) Place the three brown waste tubings in waste.

c) Connect the column between port 1 on the injection valve (7-port valve) and the UV flow cell (see Ordering information on next page for suitable columns).

d) Fill the fraction collector rack with 18 mm tubes (minimum 10) and position the white plate on the fractionation arm against the first tube.

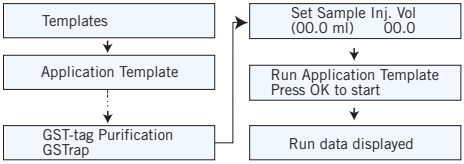
e) Connect a sample loop large enough for your sample between port 2 and 6 on the injection valve. Use a syringe to manually fill the loop.

Note: If a Superloop is needed, additional information is supplied in the instructions for Superloop.

4 Selecting Application Template and starting the method

a) Check the communication to PrimeView. At the lower right corner of the screen the text **Controlled By: prime** should be displayed.

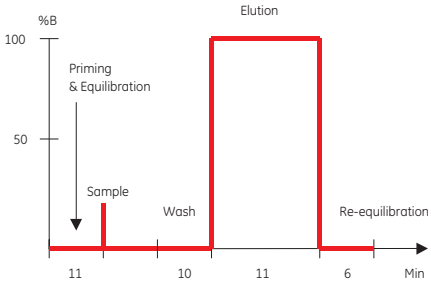
b) Use the arrow and OK buttons to move in the menu tree until you find **GST-tag Purification GSTrap**.



c) Enter the sample volume and press **OK** to start the template.

Note: If a 5 ml column is preferred, see cue card on p.36.

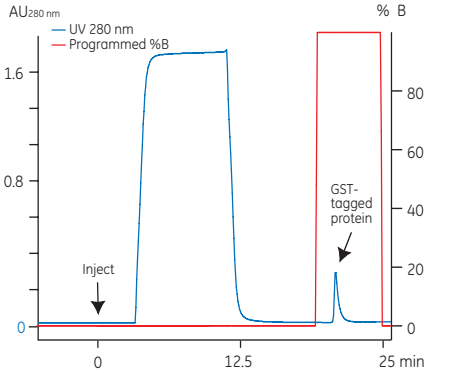
Theoretical gradient in **GST-tag Purification GSTrap** *Application Template.*



Total separation time = 37 min + sample application time

5 Typical result

Sample: Clarified homogenate of *E. coli* expressing GST-tagged protein
Column: GSTrap™ FF 1 ml
Binding buffer (A1): 20 mM sodium phosphate, 0.15 M NaCl, pH 7.3
Elution buffer (B): 50 mM Tris-HCl, 10 mM reduced glutathione, pH 8.0



Troubleshooting

High backpressure:

- *Column clogged* – Clean the column according to instructions. Make sure the sample has been centrifuged and/or filtered through a 0.45 µm filter.
- *System clogged* – Replace the column with a piece of tubing. Check pressure. If backpressure > 0.3 MPa, clean system according to manual.

No binding:

- Check that the correct column is used.
- Check that the inlet tubing from each buffer is connected to the correct inlet port.
- Check that the composition and pH of the buffers are correct.
- Check that the sample has been adjusted to binding buffer conditions.
- Check that your sample contains target protein.

No elution:

- Check that the inlet tubing from each buffer is connected to the correct inlet port.
- Check that the composition and pH of the buffers are correct.
- Use alternative elution conditions according to the column instructions.
- Check that your sample contains target protein.

Ordering information

Product	Quantity	Code No.
GSTrap FF	2 × 1 ml	17-5130-02
	5 × 1 ml	17-5130-01
	100 × 1 ml*	17-5130-05
GSTrap HP	5 × 1 ml	17-5281-01
	100 × 1 ml*	17-5281-05
GSTrap 4B	5 × 1 ml	28-4017-45
	100 × 1 ml*	28-4017-46
HiTrap Desalting	5 × 5 ml	17-1408-01
	100 × 5 ml*	11-0003-29
HiPrep 26/10 Desalting	1 (53 ml)	17-5087-01
	4 (53 ml)	17-5087-02
Superloop 10 ml	1	18-1113-83
Superloop 50 ml	1	18-1113-84
Superloop 150 ml	1	18-1023-85

* Pack size available by special order

Strep(II)-tagged protein purification

1 Preparing the buffers

- Use high purity water and chemicals.
- Filter all buffers through a 0.45 µm filter before use.

Binding buffer (port A1):
100 mM Tris-HCl, 150 mM NaCl, 1 mM EDTA, pH 8.0

Elution buffer (port B):
100 mM Tris-HCl, 150 mM NaCl, 1 mM EDTA, 2.5 mM desthiobiotin, pH 8.0

Prepare at least 500 ml of each eluent.

2 Preparing the sample

a) Adjust the sample to composition of binding buffer by:
- diluting the sample in binding buffer *or*
- by buffer exchange using HiTrap Desalting or HiPrep 26/10 Desalting.

b) Pass the sample through a 0.45 µm filter.

3 Preparing the system

a) Place the inlet tubing from port A1 (8-port valve) in the binding buffer and the tubing from port B (2-port valve) in the elution buffer.

b) Place the three brown waste tubings in waste.

c) Connect the column between port 1 on the injection valve (7-port valve) and the UV flow cell (see Ordering information on next page for suitable columns).

d) Fill the fraction collector rack with 18 mm tubes* (minimum 10) and position the white plate on the fractionation arm against the first tube.

e) Connect a sample loop large enough for your sample between port 2 and 6 on the injection valve. Use a syringe to manually fill the loop.

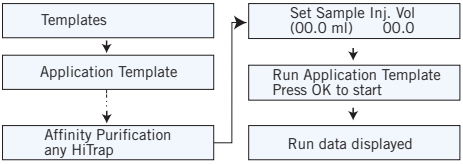
Note: If a Superloop is needed, additional information is supplied in the instructions for Superloop.

* The number of tubes to insert in the fraction collector varies with the sample volume. Fill the fraction collector with 20 tubes + one tube/ml sample. For example, if the sample volume is 10 ml, fill the fraction collector with 20 + 10 = 30 tubes. However, note that the maximum capacity of the fraction collector is 95 tubes, limiting the sample volume to 75 ml.

4 Selecting Application Template and starting the method

a) Check the communication to PrimeView. At the lower right corner of the screen the text **Controlled By: prime** should be displayed.

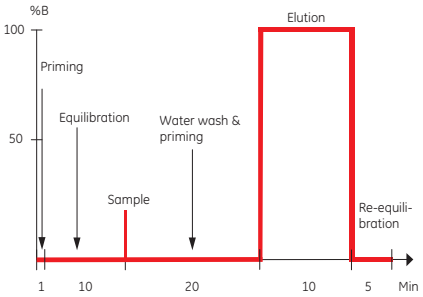
b) Use the arrow and OK buttons to move in the menu tree until you find **Affinity Purification any HiTrap**.



c) Enter the sample volume and press **OK** to start the template.

Note: If a 5 ml column is preferred, see cue card on p.36.

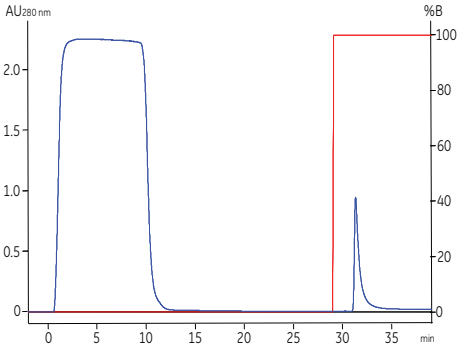
Theoretical gradient in **Affinity Purification any HiTrap** *Application Template.*



Total separation time = 47 min + sample application time

5 Typical result

Sample: Clarified lysate of *E. coli* expressing Strep(II)-tagged protein.
Column: StrepTrap™ HP 1 ml
Binding buffer (port A1): 100 mM Tris-HCl, 150 mM NaCl, 1 mM EDTA, pH 8.0
Elution buffer (port B): 100 mM Tris-HCl, 150 mM NaCl, 1 mM EDTA, 2.5 mM desthiobiotin, pH 8.0



Troubleshooting

High backpressure:

- *Column clogged* – Clean the column according to instructions. Make sure the sample has been centrifuged and/or filtered through a 0.45 µm filter.
- *System clogged* – Replace the column with a piece of tubing. Check pressure. If backpressure > 0.3 MPa, clean system according to manual.

No binding:

- Regenerate the column with 3 column volumes (CV) water, 3 CV 0.5 M NaOH, 3 CV water, 5 CV binding buffer before starting the run.
- Check that the correct column is used.
- Check that the inlet tubing from each buffer is connected to the correct inlet port.
- Check that the composition and pH of the buffers are correct.
- Check that the sample has been adjusted to the binding buffer conditions.
- Check that your sample contains target protein.

No elution:

- Check that the inlet tubing from each buffer is connected to the correct inlet port.
- Check that the composition and pH of the buffers are correct.
- Use alternative elution conditions according to the column instructions.
- Check that your sample contains target protein.

Ordering information

Product	Quantity	Code No.
StrepTrap HP	5 × 1 ml	28-9075-46
HiTrap Desalting	5 × 5 ml	17-1408-01
	100 × 5 ml*	11-0003-29
HiPrep 26/10 Desalting	1 (53 ml)	17-5087-01
	4 (53 ml)	17-5087-02
Superloop 10 ml	1	18-1113-83
Superloop 50 ml	1	18-1113-84
Superloop 150 ml	1	18-1023-85

* Pack size available by special order

MBP-tagged protein purification

1 Preparing the buffers

- Use high purity water and chemicals.
- Filter all buffers through a 0.45 µm filter before use.

Binding buffer (port A1):
20 mM Tris-HCl, 200 mM NaCl, 1 mM EDTA, 1 mM DTT, pH 7.4

Elution buffer (port B):
20 mM Tris-HCl, 200 mM NaCl, 1 mM EDTA, 1 mM DTT, 10 mM maltose, pH 7.4

Prepare at least 500 ml of each eluent.

2 Preparing the sample

a) Adjust the sample to composition of binding buffer by:
- diluting the sample in binding buffer *or*
- by buffer exchange using HiTrap Desalting or HiPrep 26/10 Desalting.

b) Pass the sample through a 0.45 µm filter.

3 Preparing the system

a) Place the inlet tubing from port A1 (8-port valve) in the binding buffer and the tubing from port B (2-port valve) in the elution buffer.

b) Place the three brown waste tubings in waste.

c) Connect the column between port 1 on the injection valve (7-port valve) and the UV flow cell (see Ordering information on next page for suitable columns).

d) Fill the fraction collector rack with 18 mm tubes* (minimum 10) and position the white plate on the fractionation arm against the first tube.

e) Connect a sample loop large enough for your sample between port 2 and 6 on the injection valve. Use a syringe to manually fill the loop.

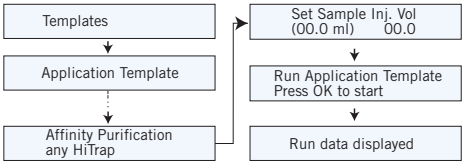
Note: If a Superloop is needed, additional information is supplied in the instructions for Superloop.

* The number of tubes to insert in the fraction collector varies with the sample volume. Fill the fraction collector with *20 tubes + one tube/ml sample*. For example, if the sample volume is 10 ml, fill the fraction collector with 20 + 10 = 30 tubes. However, note that the maximum capacity of the fraction collector is 95 tubes, limiting the sample volume to 75 ml.

4 Selecting Application Template and starting the method

a) Check the communication to PrimeView. At the lower right corner of the screen the text **Controlled By: prime** should be displayed.

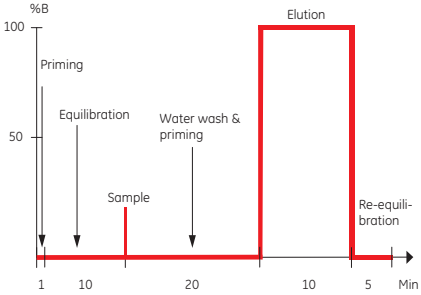
b) Use the arrow and OK buttons to move in the menu tree until you find **Affinity Purification any HiTrap**.



c) Enter the sample volume and press **OK** to start the template.

Note: If a 5 ml column is preferred, see cue card on p.36.

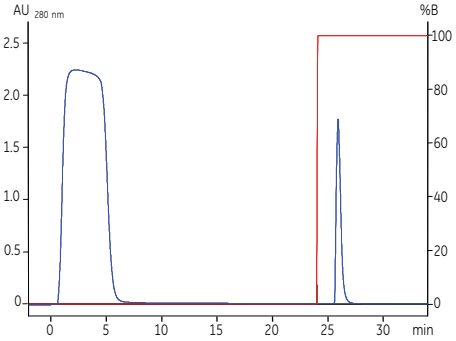
Theoretical gradient in **Affinity Purification any HiTrap** *Application Template.*



Total separation time = 47 min + sample application time

5 Typical result

Sample: Clarified lysate of *E. coli* expressing MBP-tagged protein.
Column: MBPTrap™ HP 1 ml
Binding buffer (port A1): 20 mM Tris-HCl, 200 mM NaCl, 1 mM EDTA, 1 mM DTT, pH 7.4
Elution buffer (port B): 20 mM Tris-HCl, 200 mM NaCl, 1 mM EDTA, 1 mM DTT, 10 mM maltose, pH 7.4



Troubleshooting

High backpressure:

- *Column clogged* – Clean the column according to instructions. Make sure the sample has been centrifuged and/or filtered through a 0.45 µm filter.
- *System clogged* – Replace the column with a piece of tubing. Check pressure. If backpressure > 0.3 MPa, clean system according to manual.

No binding:

- Regenerate the column with 3 column volumes (CV) water, 3 CV 0.5 M NaOH, 3 CV water, 5 CV binding buffer before starting the run.
- Check that the correct column is used.
- Check that the inlet tubing from each buffer is connected to the correct inlet port.
- Check that the composition and pH of the buffers are correct.
- Check that the sample has been adjusted to the binding buffer conditions.
- Check that your sample contains target protein.

No elution:

- Check that the inlet tubing from each buffer is connected to the correct inlet port.
- Check that the composition and pH of the buffers are correct.
- Use alternative elution conditions according to the column instructions.
- Check that your sample contains target protein.

Ordering information

Product	Quantity	Code No.
MBPTrap HP	5 × 1 ml	28-9187-78
HiTrap Desalting	5 × 5 ml	17-1408-01
	100 × 5 ml*	11-0003-29
HiPrep 26/10 Desalting	1 (53 ml)	17-5087-01
	4 (53 ml)	17-5087-02
Superloop 10 ml	1	18-1113-83
Superloop 50 ml	1	18-1113-84
Superloop 150 ml	1	18-1023-85

* Pack size available by special order

MAb purification, step elution

1 Preparing the buffers

- Use high purity water and chemicals.
- Filter all buffers thtough a 0.45 µm filter before use.

Binding buffer (port A1):
20 mM sodium phosphate, pH 7.0*

Elution buffer (port B):
0.1 M glycine-HCl, pH 3.0

Prepare at least 500 ml of each eluent.

* With some antibodies, e.g. mouse IgG_1 it might be necessary to add NaCl up to 3 M in the binding buffer, to achieve efficient binding when using HiTrap Protein A HP and HiTrap rProtein A FF.

2 Preparing the sample

a) Adjust the sample to composition of binding buffer by:
- diluting the sample with binding buffer *or*
- by buffer exchange using HiTrap Desalting or HiPrep 26/10 Desalting.

b) Pass the sample through a 0.45 µm filter.

3 Preparing the system

a) Place the inlet tubing from port A1 (8-port valve) in the binding buffer and the tubing from port B (2-port valve) in the elution buffer.

b) Place the three brown waste tubings in waste.

c) Connect the column between port 1 on the injection valve (7-port valve) and the UV flow cell (see Ordering information on next page for suitable columns).

d) Fill the fraction collector rack with 18 mm tubes (minimum 10) and position the white plate on the fractionation arm against the first tube.

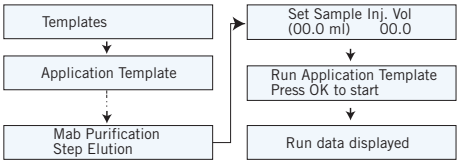
e) Connect a sample loop large enough for your sample between port 2 and 6 on the injection valve. Use a syringe to manually fill the loop.

Note: If a Superloop is needed, additional information is supplied in the instructions for Superloop.

4 Selecting Application Template and starting the method

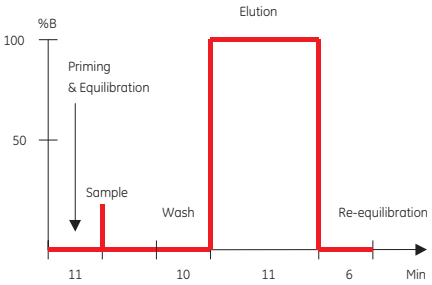
a) Check the communication to PrimeView. At the lower right corner of the screen the text **Controlled By: prime** should be displayed.

b) Use the arrow and OK buttons to move in the menu tree until you find **Mab Purification Step Elution**.



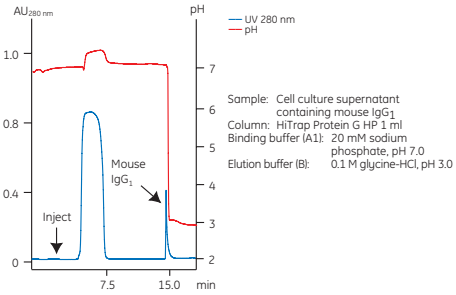
c) Enter the sample volume and press **OK** to start the template.
Note: If a 5 ml column is preferred, see cue card on p.36.

Theoretical gradient in **Mab Purification Step Elution** *Application Template.*



Total separation time = 37 min + sample application time

5 Typical result



Troubleshooting

High backpressure:

- *Column clogged* – Clean the column according to instructions. Make sure the sample has been centrifuged and/or filtered through a 0.45 µm filter.
- *System clogged* – Replace the column with a piece of tubing. Check pressure. If backpressure > 0.3 MPa, clean system according to manual.

No binding:

- Check that the correct column is used.
- Check that the inlet tubing from each buffer is connected to the correct inlet port.
- Check that the composition and pH of the buffers are correct.
- Check that the sample has been adjusted to binding buffer conditions.
- Check that your sample contains target protein.

No elution:

- Check that the inlet tubing from each buffer is connected to the correct inlet port.
- Check that the composition and pH of the buffers are correct.
- Use alternative elution conditions according to the column instructions.
- Check that your sample contains target protein.

Ordering information

Product	Quantity	Code No.
HiTrap Protein G HP	2 × 1 ml	17-0404-03
	5 × 1 ml	17-0404-01
HiTrap Protein A HP	2 × 1 ml	17-0402-03
	5 × 1 ml	17-0402-01
HiTrap rProtein A FF	2 × 1 ml	17-5079-02
	5 × 1 ml	17-5079-01
HiTrap MabSelect SuRe™	5 × 1 ml	11-0034-93
HiTrap MabSelect™	5 × 1 ml	28-4082-53
HiTrap MabSelect Xtra™	5 × 1 ml	28-4082-58
HiTrap Desalting	5 × 5 ml	17-1408-01
	100 × 5 ml*	11-0003-29
HiPrep 26/10 Desalting	1 (53 ml)	17-5087-01
	4 (53 ml)	17-5087-02
Superloop 10 ml	1	18-1113-83
Superloop 50 ml	1	18-1113-84
Superloop 150 ml	1	18-1023-85

* Pack size available by special order

MAb purification, gradient elution

1 Preparing the buffers

- Use high purity water and chemicals.
- Filter all buffers through a 0.45 µm filter before use.

Binding buffer (port A1):
100 mM sodium phosphate, 100 mM sodium citrate, pH 7.0*

Elution buffer (port B):
100 mM sodium phosphate, 100 mM sodium citrate, pH 3.0

Prepare at least 500 ml of each eluent.

* With some antibodies, e.g. mouse IgG_1 it might be necessary to add NaCl up to 3 M in the binding buffer, to achieve efficient binding when using HiTrap Protein A HP and HiTrap rProtein A FF.

2 Preparing the sample

a) Adjust the sample to composition of binding buffer by:
- diluting the sample in binding buffer *or*
- by buffer exchange using HiTrap Desalting or HiPrep 26/10 Desalting.

b) Pass the sample through a 0.45 µm filter.

3 Preparing the system

a) Place the inlet tubing from port A1 (8-port valve) in the binding buffer and the tubing from port B (2-port valve) in the elution buffer.

b) Place the three brown waste tubings in waste.

c) Connect the column between port 1 on the injection valve (7-port valve) and the UV flow cell (see Ordering information on next page for suitable columns).

d) Fill the fraction collector rack with 18 mm tubes (minimum 40) and position the white plate on the fractionation arm against the first tube.

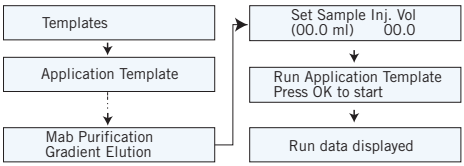
e) Connect a sample loop large enough for your sample between port 2 and 6 on the injection valve. Use a syringe to manually fill the loop.

Note: If a Superloop is needed, additional information is supplied in the instructions for Superloop.

4 Selecting Application Template and starting the method

a) Check the communication to PrimeView. At the lower right corner of the screen the text **Controlled By: prime** should be displayed.

b) Use the arrow and OK buttons to move in the menu tree until you find **Mab Purification Gradient Elution**.



c) Enter the sample volume and press **OK** to start the template.

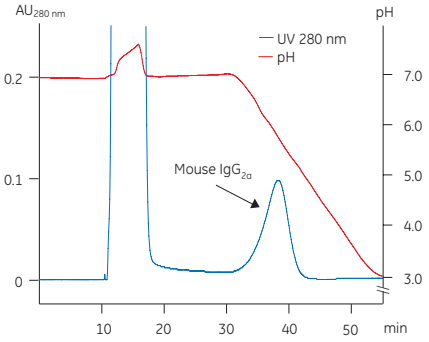
Note: If a 5 ml column is preferred, see cue card on p.36.

Theoretical gradient in **Mab Purification Gradient Elution** *Application Template.*

Total separation time = 63 min + sample application time

5 Typical result

Sample: Cell culture supernatant containing mouse IgG_{2a}
Column: HiTrap Protein A HP 1 ml
Binding buffer (A1): 0.1 M sodium phosphate, 0.1 M sodium citrate, pH 7.0
Elution buffer (B): 0.1 M sodium phosphate, 0.1 M sodium citrate, pH 3.0



Troubleshooting

High backpressure:

- *Column clogged* – Clean the column according to instructions. Make sure the sample has been centrifuged and/or filtered through a 0.45 µm filter.
- *System clogged* – Replace the column with a piece of tubing. Check pressure. If backpressure > 0.3 MPa, clean system according to manual.

No binding:

- Check that the correct column is used.
- Check that the inlet tubing from each buffer is connected to the correct inlet port.
- Check that the composition and pH of the buffers are correct.
- Check that the sample has been adjusted to binding buffer conditions.
- Check that your sample contains target protein.

No elution:

- Check that the inlet tubing from each buffer is connected to the correct inlet port.
- Check that the composition and pH of the buffers are correct.
- Use alternative elution conditions according to the column instructions.
- Check that your sample contains target protein.

Ordering information

Product	Quantity	Code No.
HiTrap Protein G HP	2 × 1 ml	17-0404-03
	5 × 1 ml	17-0404-01
HiTrap Protein A HP	2 × 1 ml	17-0402-03
	5 × 1 ml	17-0402-01
HiTrap rProtein A FF	2 × 1 ml	17-5079-02
	5 × 1 ml	17-5079-01
HiTrap MabSelect SuRe	5 × 1 ml	11-0034-93
HiTrap MabSelect	5 × 1 ml	28-4082-53
HiTrap MabSelect Xtra	5 × 1 ml	28-4082-58
HiTrap Desalting	5 × 5 ml	17-1408-01
	100 × 5 ml*	11-0003-29
HiPrep 26/10 Desalting	1 (53 ml)	17-5087-01
	4 (53 ml)	17-5087-02
Superloop 10 ml	1	18-1113-83
Superloop 50 ml	1	18-1113-84
Superloop 150 ml	1	18-1023-85

* Pack size available by special order

IgM purification

1 Preparing the buffers

- Use high purity water and chemicals.
- Filter all buffers through a 0.45 µm filter before use.

Binding buffer (port A1):
20 mM sodium phosphate, 0.8 M $(NH_4)_2SO_4$, pH 7.5

Elution buffer 1 (port B):
20 mM sodium phosphate, pH 7.5

Regeneration buffer 2 (port A2):
20 mM sodium phosphate, 30% isopropanol, pH 7.5

Prepare at least 500 ml of each eluent.

2 Preparing the sample

a) Adjust the sample to composition of binding buffer by:
- diluting the sample in binding buffer *or*
- by buffer exchange using HiTrap Desalting or HiPrep 26/10 Desalting.

b) Pass the sample through a 0.45 µm filter.

3 Preparing the system

a) Place each inlet tubing from port A (8-port valve) in eluents as given above and the tubing from port B (2-port valve) in elution buffer 1.

b) Place the three brown waste tubings in waste.

c) Connect the column between port 1 on the injection valve (7-port valve) and the UV flow cell (see Ordering information on next page for suitable columns).

d) Fill the fraction collector rack with 18 mm tubes (minimum 15) and position the white plate on the fractionation arm against the first tube.

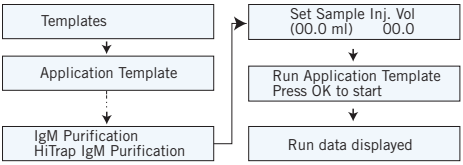
e) Connect a sample loop large enough for your sample between port 2 and 6 on the injection valve. Use a syringe to manually fill the loop.

Note: If a Superloop is needed, additional information is supplied in the instructions for Superloop.

4 Selecting Application Template and starting the method

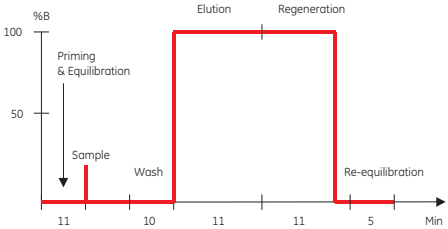
a) Check the communication to PrimeView. At the lower right corner of the screen the text **Controlled By: prime** should be displayed.

b) Use the arrow and OK buttons to move in the menu tree until you find **IgM Purification HiTrap IgM Purification**.



c) Enter the sample volume and press **OK** to start the template.

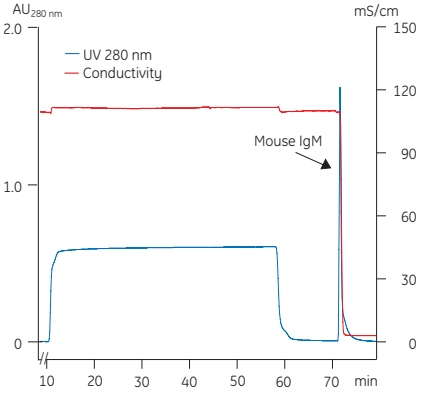
Theoretical gradient in **IgM Purification HiTrap IgM Purification** *Application Template.*



Total separation time = 48 min + sample application time

5 Typical result

Sample: Hybridoma cell culture containing IgM
Column: HiTrap IgM Purification HP 1 ml
Binding buffer (A1): 20 mM sodium phosphate, 0.8 M $(NH_4)_2SO_4$, pH 7.5
Elution buffer 1 (B): 20 mM sodium phosphate, pH 7.5
Elution buffer 2 (A2): 20 mM sodium phosphate, 30% isopropanol, pH 7.5



Troubleshooting

High backpressure:

- *Column clogged* – Clean the column according to instructions. Make sure the sample has been centrifuged and/or filtered through a 0.45 µm filter.
- *System clogged* – Replace the column with a piece of tubing. Check pressure. If backpressure > 0.3 MPa, clean system according to manual.

No binding:

- Check that the correct column is used.
- Check that the inlet tubing from each buffer is connected to the correct inlet port.
- Check that the composition and pH of the buffers are correct.
- Check that the sample has been adjusted to binding buffer conditions.
- Check that your sample contains target protein.

No elution:

- Check that the inlet tubing from each buffer is connected to the correct inlet port.
- Check that the composition and pH of the buffers are correct.
- Use alternative elution conditions according to the column instructions.
- Check that your sample contains target protein.

Ordering information

Product	Quantity	Code No.
HiTrap IgM Purification HP	5 × 1 ml	17-5110-01
HiTrap Desalting	5 × 5 ml	17-1408-01
	100 × 5 ml*	11-0003-29
HiPrep 26/10 Desalting	1 (53 ml)	17-5087-01
	4 (53 ml)	17-5087-02
Superloop 10 ml	1	18-1113-83
Superloop 50 ml	1	18-1113-84
Superloop 150 ml	1	18-1023-85

* Pack size available by special order

Albumin removal

1 Preparing the buffers

- Use high purity water and chemicals.
- Filter all buffers through a 0.45 µm filter before use.

Binding buffer (port A1):
20 mM sodium phosphate, pH 7.0

Elution buffer (port B):
20 mM sodium phosphate, 2.0 M NaCl, pH 7.0

Prepare at least 500 ml of each eluent.

2 Preparing the sample

a) Adjust the sample to composition of binding buffer by:
- diluting the sample in binding buffer *or*
- by buffer exchange using HiTrap Desalting or HiPrep 26/10 Desalting.

b) Pass the sample through a 0.45 µm filter.

3 Preparing the system

a) Place the inlet tubing from port A1 (8-port valve) in the binding buffer and the tubing from port B (2-port valve) in the elution buffer.

b) Place the three brown waste tubings in waste.

c) Connect the column between port 1 on the injection valve (7-port valve) and the UV flow cell (see Ordering information on next page for suitable columns).

d) Fill the fraction collector rack with 18 mm tubes (minimum 20) and position the white plate on the fractionation arm against the first tube.

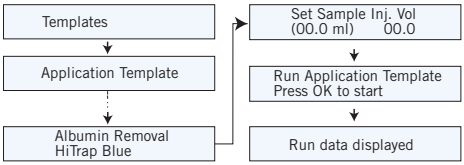
e) Connect a sample loop large enough for your sample between port 2 and 6 on the injection valve. Use a syringe to manually fill the loop.

Note: If a Superloop is needed, additional information is supplied in the instructions for Superloop.

4 Selecting Application Template and starting the method

a) Check the communication to PrimeView. At the lower right corner of the screen the text **Controlled By: prime** should be displayed.

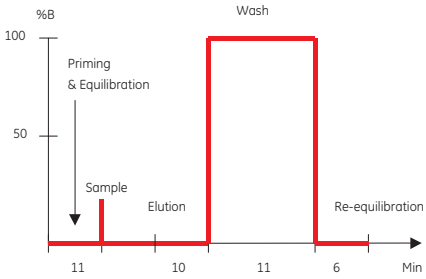
b) Use the arrow and OK buttons to move in the menu tree until you find **Albumin Removal HiTrap Blue**.



c) Enter the sample volume and press **OK** to start the template.

Note: If a 5 ml column is preferred, see cue card on p.36.

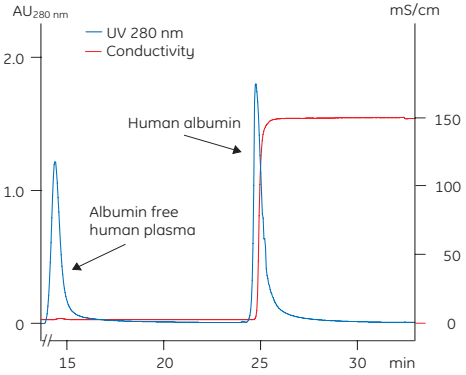
Theoretical gradient in **Albumin Removal HiTrap Blue** *Application Template.*



Total separation time = 37 min + sample application time

5 Typical result

Sample: Human plasma, buffer exchanged to binding buffer using HiTrap Desalting
Column: HiTrap Blue HP 1 ml
Binding buffer (A1): 20 mM sodium phosphate, pH 7.0
Elution buffer (B): 20 mM sodium phosphate, 2.0 M NaCl, pH 7.0



Troubleshooting

High backpressure:

- *Column clogged* – Clean the column according to instructions. Make sure the sample has been centrifuged and/or filtered through a 0.45 µm filter.
- *System clogged* – Replace the column with a piece of tubing. Check pressure. If backpressure > 0.3 MPa, clean system according to manual.

No binding:

- Check that the correct column is used.
- Check that the inlet tubing from each buffer is connected to the correct inlet port.
- Check that the composition and pH of the buffers are correct.
- Check that the sample has been adjusted to binding buffer conditions.
- Check that your sample contains target protein.

No elution:

- Check that the inlet tubing from each buffer is connected to the correct inlet port.
- Check that the composition and pH of the buffers are correct.
- Use alternative elution conditions according to the column instructions.
- Check that your sample contains target protein.

Ordering information

Product	Quantity	Code No.
HiTrap Blue HP	5 × 1 ml	17-0412-01
HiTrap Desalting	5 × 5 ml	17-1408-01
	100 × 5 ml*	11-0003-29
HiPrep 26/10 Desalting	1 (53 ml)	17-5087-01
	4 (53 ml)	17-5087-02
Superloop 10 ml	1	18-1113-83
Superloop 50 ml	1	18-1113-84
Superloop 150 ml	1	18-1023-85

* Pack size available by special order

Removal of trypsin-like serine proteases

1 Preparing the buffers

- Use high purity water and chemicals.
- Filter all buffers through a 0.45 µm filter before use.

Binding buffer (port A1):
50 mM Tris-HCl, 0.5 M NaCl, pH 7.4

Elution buffer (port B):
50 mM glycine-HCl buffer, pH 3.0

Prepare at least 500 ml of each eluent.

2 Preparing the sample

a) Adjust the sample to composition of binding buffer by:
   - diluting the sample in binding buffer or
   - by buffer exchange using HiTrap Desalting or HiPrep 26/10 Desalting.

b) Pass the sample through a 0.45 µm filter.

3 Preparing the system

a) Place the inlet tubing from port A1 (8-port valve) in the binding buffer and the tubing from port B (2-port valve) in the elution buffer.

b) Place the three brown waste tubings in waste.

c) Connect the column between port 1 on the injection valve (7-port valve) and the UV flow cell (see Ordering information on next page for suitable columns).

d) Fill the fraction collector rack with 18 mm tubes* (minimum 40) and position the white plate on the fractionation arm against the first tube.

e) Connect a sample loop large enough for your sample between port 2 and 6 on the injection valve. Use a syringe to manually fill the loop.

   Note: If a Superloop is needed, additional information is supplied in the instructions for Superloop.

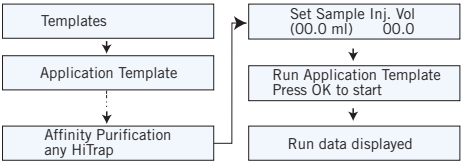
4 Selecting Application Template and starting the method

a) Check the communication to PrimeView. At the lower right corner of the screen the text **Controlled By: prime** should be displayed.

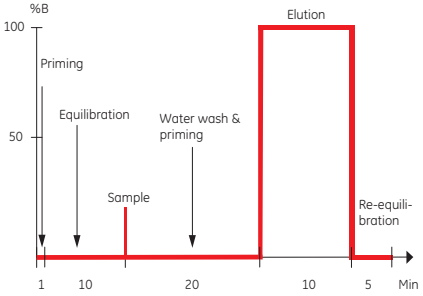
b) Use the arrow and OK buttons to move in the menu tree until you find **Affinity Purification any HiTrap**.

c) Enter the sample volume and press **OK** to start the template.

   Note: If a 5 ml column is preferred see cue card on p.36.



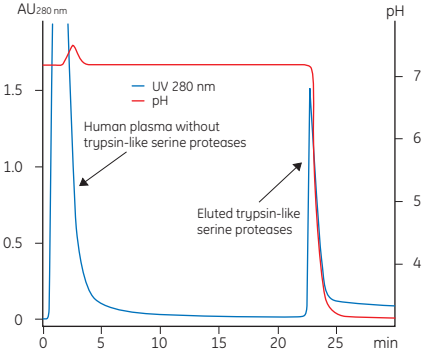
Theoretical gradient in **Affinity Purification any HiTrap** *Application Template.*



Total separation time = 47 min + sample application time

5 Typical result

Sample: Human plasma
Column: HiTrap Benzamidine FF (high sub) 1 ml
Binding buffer (port A1): 50 mM Tris-HCl, 0.5 M NaCl, pH 7.4
Elution buffer (port B): 50 mM Glycine-HCl, pH 3.0



Troubleshooting

High backpressure:

- *Column clogged* – Clean the column according to instructions. Make sure the sample has been centrifuged and/or filtered through a 0.45 µm filter.
- *System clogged* – Replace the column with a piece of tubing. Check pressure. If backpressure > 0.3 MPa, clean system according to manual.

No binding:

- Check that the correct column is used.
- Check that the inlet tubing from each buffer is connected to the correct inlet port.
- Check that the composition and pH of the buffers are correct.
- Check that the sample has been adjusted to binding buffer conditions.
- Check that your sample contains target protein.

No elution:

- Check that the inlet tubing from each buffer is connected to the correct inlet port.
- Check that the composition and pH of the buffers are correct.
- Use alternative elution conditions according to the column instructions.
- Check that your sample contains target protein.

Ordering information

Product	Quantity	Code No.
HiTrap Benzamidine FF (high sub)	2 × 1 ml	17-5143-02
	5 × 1 ml	17-5143-01
Superloop 10 ml	1	18-1113-83
Superloop 50 ml	1	18-1113-84
Superloop 150 ml	1	18-1023-85

Anion exchange

1 Preparing the buffers

- Use high purity water and chemicals.
- Filter all buffers through a 0.45 µm filter before use.

Start buffer (port A1):
20 mM Tris-HCl, pH 8.0

Elution buffer (port B):
20 mM Tris-HCl, 1.0 M NaCl, pH 8.0

Prepare at least 500 ml of each buffer.

Alternative buffers:

Start buffer (port A1): 20 mM Glycin-NaOH, pH 9.5

Elution buffer (port B): 20 mM Glycin-NaOH, 1.0 M NaCl, pH 9.5

Start buffer (port A1): 20 mM bis-Tris, pH 6.5

Elution buffer (port B): 20 mM bis-Tris, 1.0 M NaCl, pH 6.5

2 Preparing the sample

a) Adjust the sample to composition of start buffer by:
- diluting the sample in binding buffer *or*
- by buffer exchange using HiTrap Desalting or HiPrep 26/10 Desalting.

b) Pass the sample through a 0.45 µm filter.

3 Preparing the system

a) Place the inlet tubing from port A1 (8-port valve) in the binding buffer and the tubing from port B (2-port valve) in the elution buffer.

b) Place the three brown waste tubings in waste.

c) Connect the column between port 1 on the injection valve (7-port valve) and the UV flow cell (see Ordering information on next page for suitable columns).

d) Fill the fraction collector rack with 18 mm tubes (minimum 40) and position the white plate on the fractionation arm against the first tube.

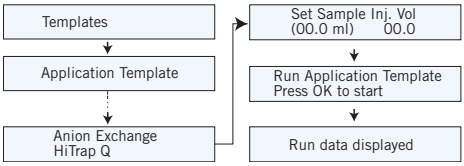
e) Connect a sample loop large enough for your sample between port 2 and 6 on the injection valve. Use a syringe to manually fill the loop.

Note: If a Superloop is needed, additional information is supplied in the instructions for Superloop.

4 Selecting Application Template and starting the method

a) Check the communication to PrimeView. At the lower right corner of the screen the text **Controlled By: prime** should be displayed.

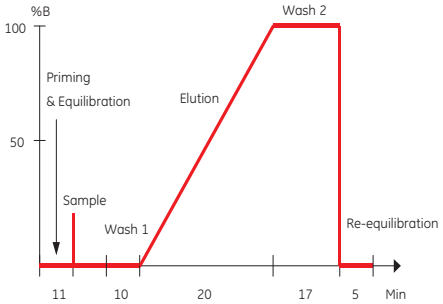
b) Use the arrow and OK buttons to move in the menu tree until you find **Anion Exchange HiTrap Q**.



c) Enter the sample volume and press **OK** to start the template.

Note: If a 5 ml column is preferred see cue card on p.36.

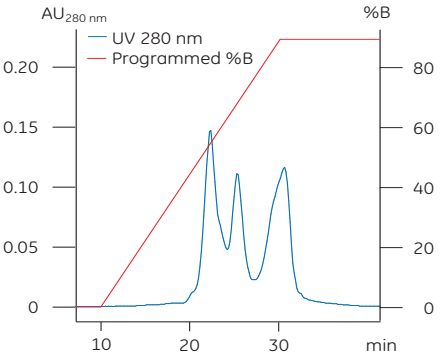
Theoretical gradient in **Anion Exchange HiTrap Q** *Application Template.*



Total separation time = 63 min + sample application time

5 Typical result

Sample: Protein mix containing transferrin, ovalbumin and ß-lactoglobulin in start buffer
Column: HiTrap Q HP 1 ml
Start buffer (A1): 20 mM Tris-HCl, pH 8.0
Elution buffer (B): 20 mM Tris-HCl, 1.0 M NaCl, pH 8.0



Troubleshooting

High backpressure:

- *Column clogged* – Clean the column according to instructions. Make sure the sample has been centrifuged and/or filtered through a 0.45 µm filter.
- *System clogged* – Replace the column with a piece of tubing. Check pressure. If backpressure > 0.3 MPa, clean system according to manual.

No binding:

- Check that the correct column is used.
- Check that the inlet tubing from each buffer is connected to the correct inlet port.
- Check that the composition and pH of the buffers are correct. If the compostion and pH of the buffers are correct, but there is still no binding:
  a) If the protein of interest does not bind to the column, the pH should be increased.
  b) If it still not binds, it is advisable to try a cation exchanger (see cue card: Cation exchange).
  c) If the protein of interest binds to the column but the separation is poor, the pH should be decreased.
- Check that the sample has been adjusted to start buffer conditions.
- Check that your sample contains target protein.

No elution:

- Check that the inlet tubing from each buffer is connected to the correct inlet port.
- Check that the composition and pH of the buffers are correct.
- Use alternative elution conditions according to the column instructions.
- Check that your sample contains target protein.

Ordering information

Product	Quantity	Code No.
HiTrap Q HP	5 × 1 ml	17-1153-01
HiTrap Q FF	5 × 1 ml	17-5053-01
HiTrap DEAE FF	5 × 1 ml	17-5055-01
HiTrap ANX FF (high sub)	5 × 1 ml	17-5162-01
HiTrap Q XL	5 × 1 ml	17-5158-01
HiTrap Capto™ Q	5 × 1 ml	11-0013-02
HiTrap Capto DEAE	5 × 1 ml	28-9165-37
HiTrap Capto adhere	5 × 1 ml	28-4058-44
HiTrap IEX Selection Kit	7 × 1 ml	17-6002-33
HiTrap Desalting	5 × 5 ml	17-1408-01
	100 × 5 ml*	11-0003-29
HiPrep 26/10 Desalting	1 (53 ml)	17-5087-01
	4 (53 ml)	17-5087-02
Superloop 10 ml	1	18-1113-83
Superloop 50 ml	1	18-1113-84
Superloop 150 ml	1	18-1023-85

* Pack size available by special order

Cation exchange

1 Preparing the buffers

- Use high purity water and chemicals.
- Filter all buffers thtough a 0.45 µm filter before use.

Start buffer (port A1):
50 mM sodium acetate, pH 5.5

Elution buffer (port B):
50 mM sodium acetate, 1.0 M NaCl, pH 5.5

Prepare at least 500 ml of each eluent.

Alternative buffers:

Start buffer (port A1): 20 mM sodium phosphate, pH 7.0

Elution buffer (port B): 20 mM sodium phosphate, 1.0 M NaCl, pH 7.0

Start buffer (port A1): 20 mM sodium citrate, pH 3.0

Elution buffer (port B): 20 mM sodium citrate, 1.0 M NaCl, pH 3.0

2 Preparing the sample

a) Adjust the sample to composition of binding buffer by:
- diluting the sample in binding buffer *or*
- by buffer exchange using HiTrap Desalting or HiPrep 26/10 Desalting.

b) Pass the sample through a 0.45 µm filter.

3 Preparing the system

a) Place the inlet tubing from port A1 (8-port valve) in the binding buffer and the tubing from port B (2-port valve) in the elution buffer.

b) Place the three brown waste tubings in waste.

c) Connect the column between port 1 on the injection valve (7-port valve) and the UV flow cell (see Ordering information on next page for suitable columns).

d) Fill the fraction collector rack with 18 mm tubes (minimum 40) and position the white plate on the fractionation arm against the first tube.

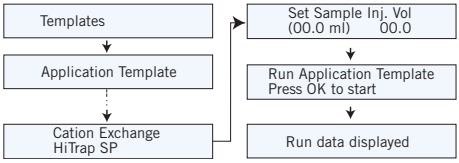
e) Connect a sample loop large enough for your sample between port 2 and 6 on the injection valve. Use a syringe to manually fill the loop.

Note: If a Superloop is needed, additional information is supplied in the instructions for Superloop.

4 Selecting Application Template and starting the method

a) Check the communication to PrimeView. At the lower right corner of the screen the text **Controlled By: prime** should be displayed.

b) Use the arrow and OK buttons to move in the menu tree until you find **Cation Exchange HiTrap SP**.



c) Enter the sample volume and press **OK** to start the template.

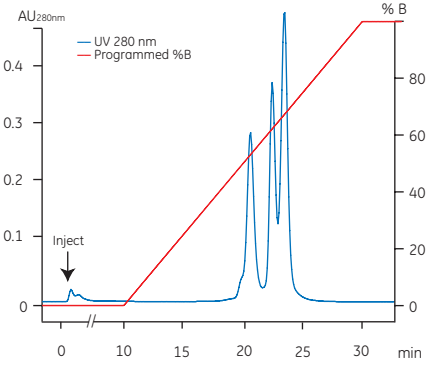
Note: If a 5 ml column is preferred, see cue card on p.36.

Theoretical gradient in **Cation Exchange HiTrap SP** *Application Template.*

Total separation time = 63 min + sample application time

5 Typical result

Sample: Protein mix containing chymotrypsinogen A, cytochrome C and lysozyme in start buffer
Column: HiTrap SP HP 1 ml
Start buffer (A1): 20 mM sodium acetate, pH 5.5
Elution buffer (B): 20 mM sodium acetate, 1.0 M NaCl, pH 5.5



Troubleshooting

High backpressure:

- *Column clogged* – Clean the column according to instructions. Make sure the sample has been centrifuged and/or filtered through a 0.45 µm filter.
- *System clogged* – Replace the column with a piece of tubing. Check pressure. If backpressure > 0.3 MPa, clean system according to manual.

No binding:

- Check that the correct column is used.
- Check that the inlet tubing from each buffer is connected to the correct inlet port.
- Check that the composition and pH of the buffers are correct. If the compostion and pH of the buffers are correct, but there is still no binding:
  a) If the protein of interest does not bind to the column, the pH should be decreased.
  b) If it still not binds, it is advisable to try an anion exchanger (see cue card: Anion exchange).
  c) If the protein of interest binds to the column but the separation is poor, the pH should be increased.
- Check that the sample has been adjusted to start buffer conditions.
- Check that your sample contains target protein.

No elution:

- Check that the inlet tubing from each buffer is connected to the correct inlet port.
- Check that the composition and pH of the buffers are correct.
- Use alternative elution conditions according to the column instructions.
- Check that your sample contains target protein.

Ordering information

Product	Quantity	Code No.
HiTrap SP HP	5 × 1 ml	17-1151-01
HiTrap SP FF	5 × 1 ml	17-5054-01
HiTrap CM FF	5 × 1 ml	17-5056-01
HiTrap SP XL	5 × 1 ml	17-5160-01
HiTrap Capto S	5 × 1 ml	17-5441-22
HiTrap Capto MMC	5 × 1 ml	11-0032-73
HiTrap IEX Selection Kit	7 × 1 ml	17-6002-33
HiTrap Desalting	5 × 5 ml	17-1408-01
	100 × 5 ml*	11-0003-29
HiPrep 26/10 Desalting	1 (53 ml)	17-5087-01
	4 (53 ml)	17-5087-02
Superloop 10 ml	1	18-1113-83
Superloop 50 ml	1	18-1113-84
Superloop 150 ml	1	18-1023-85

* Pack size available by special order

Method Templates value table

The Method Templates provides an easy and fast way to build up a method. By setting defined parameters (and volumes) for each part of the chromatographic phase a method is built up.

This table contains suggested values for a selection of columns which enables a fast start. The values can then be optimized to suit the specific application.

Code No.	Column vol. ml	Pressure* MPa	Flow ml/min	Frac.vol ml	Equil.vol ml	Wash1 vol. ml	Elu.vol ml	Wash2 ml
Affinity								
17-0402-01	HiTrap Protein A HP, 1 ml	0.5	1	0.5	5	10	5	0
17-0403-01	HiTrap Protein A HP, 5 ml	0.5	5	2.5	25	50	25	0
17-5079-01	HiTrap rProtein A FF, 1 ml	0.5	1	0.5	5	10	5	0
17-5080-01	HiTrap rProtein A FF, 5 ml	0.5	5	2.5	25	50	25	0
17-0404-01	HiTrap Protein G HP, 1 ml	0.5	1	0.5	5	10	5	0
17-0405-01	HiTrap Protein G HP, 5 ml	0.5	5	2.5	25	50	25	0
11-0034-93	HiTrap MabSelect SuRe, 1 ml	0.5	1	0.5	5	10	5	0
11-0034-94	HiTrap MabSelect SuRe, 5 ml	0.5	5	2.5	25	50	25	0
28-4082-53	HiTrap MabSelect, 1 ml	0.5	1	0.5	5	10	5	0
28-4082-55	HiTrap MabSelect, 5 ml	0.5	5	2.5	25	50	25	0
28-4082-58	HiTrap MabSelect Xtra, 1 ml	0.5	1	0.5	5	10	5	0
28-4082-60	HiTrap MabSelect Xtra, 5 ml	0.5	5	2.5	25	50	25	0
17-5110-01	HiTrap IgM Purification, 1 ml	0.5	1	0.5	5	15	15	10
17-5111-01	HiTrap IgY Purification, 5 ml	0.5	5	2.5	25	50	50	40
17-5247-01	HisTrap HP, 1 ml	0.5	1	0.5	5	20	5	0
17-5248-02	HisTrap HP, 5 ml	0.5	5	2.5	25	100	25	0
17-5319-01	HisTrap FF, 1 ml	0.5	1	0.5	5	20	5	0
17-5255-01	HisTrap FF, 5 ml	0.5	5	2.5	25	100	25	0
11-0004-58	HisTrap FF crude, 1 ml	0.5	1	0.5	5	20	5	0
17-5286-01	HisTrap FF crude, 5 ml	0.5	5	2.5	25	100	25	0
17-5281-01	GSTrap HP, 1 ml	0.5	0.3	0.5	5	20	5	0
17-5282-01	GSTrap HP, 5 ml	0.5	1.5	2.5	25	100	25	0
17-5130-01	GSTrap FF, 1 ml	0.5	0.3	0.5	5	20	5	0
17-5131-01	GSTrap FF, 5 ml	0.5	1.5	2.5	25	100	25	0
28-4017-45	GSTrap 4B, 1 ml	0.5	0.3	0.5	5	20	5	0
28-4017-47	GSTrap 4B, 5 ml	0.5	1.5	2.5	25	100	25	0
28-9187-78	MBPTrap HP, 1 ml	0.5	1	0.5	5	10	5	0
28-9187-79	MBPTrap HP, 5 ml	0.5	5	2.5	25	50	25	0
28-9075-46	StrepTrap HP, 1 ml	0.5	1	0.5	5	10	5	0
28-9075-47	StrepTrap HP, 5 ml	0.5	5	2.5	25	50	25	0
17-0920-03	HiTrap IMAC HP, 1 ml	0.5	1	0.5	5	20	5	0
17-0920-05	HiTrap IMAC HP, 5 ml	0.5	5	2.5	25	100	25	0
17-0921-02	HiTrap IMAC FF, 1 ml	0.5	1	0.5	5	20	5	0
17-0921-04	HiTrap IMAC FF, 5 ml	0.5	5	2.5	25	100	25	0
17-0408-01	HiTrap Chelating HP, 1 ml	0.5	1	0.5	5	20	5	0
17-0409-03	HiTrap Chelating HP, 5 ml	0.5	5	2.5	25	100	25	0
17-0412-01	HiTrap Blue HP, 1 ml	0.5	1	0.5	5	10	5	0
17-0413-01	HiTrap Blue HP, 5 ml	0.5	5	2.5	25	50	25	0

The table continues on next page.

Code No.	Column vol. ml	Pressure* MPa	Flow ml/min	Frac.vol ml	Equil.vol ml	Wash1 vol. ml	Elu.vol ml	Wash2 ml
17-0406-01	HiTrap Heparin HP, 1 ml	0.5	1	0.5	5	10	5	0
17-0407-03	HiTrap Heparin HP, 5 ml	0.5	5	2.5	25	50	25	0
17-5112-01	HiTrap Streptavidin HP, 1 ml	0.5	1	0.5	10	10	10	0
17-5143-01	HiTrap Benzamidine FF (high sub), 1 ml	0.5	1	0.5	5	20	5	0
17-5144-01	HiTrap Benzamidine FF (high sub), 5 ml	0.5	5	2.5	25	100	25	0
17-5189-01	HiPrep 16/10 Heparin FF	0.5	5	5	100	200	100	0
17-0921-06	HiPrep IMAC FF 16/10	0.5	5	5	100	200	100	0
17-5234-01	GSTPrep FF 16/10	0.5	1.5	5	100	400	100	0
17-5256-01	HisPrep™ FF 16/10	0.5	1.5	5	100	400	100	0
Buffer exchange								
17-1408-01	HiTrap Desalting, 5 ml	0.5	5	0.5	15	0	7	–
	HiTrap Desalting, 2 × 5 ml	0.5	5	1	30	0	14	0
17-5087-01	HiPrep 26/10 Desalting	0.35	10	2.5	100	0	80	–
Ion exchange								
17-6002-33	HiTrap IEX Selection Kit, 1 ml	0.5	1	1	5	2	20	5
17-1153-01	HiTrap Q HP, 1 ml	0.5	1	1	5	2	20	5
17-1154-01	HiTrap Q HP, 5 ml	0.5	5	5	25	10	100	25
17-5053-01	HiTrap Q FF, 1 ml	0.5	1	1	5	2	20	5
17-5156-01	HiTrap Q FF, 5 ml	0.5	5	5	25	10	100	25
17-5055-01	HiTrap DEAE FF, 1 ml	0.5	1	1	5	2	20	5
17-5154-01	HiTrap DEAE FF, 5 ml	0.5	5	5	25	10	100	25
17-5162-01	HiTrap ANX FF (high sub), 1 ml	0.5	1	1	5	2	20	5
17-5163-01	HiTrap ANX FF (high sub), 5 ml	0.5	5	5	25	10	100	25
17-5158-01	HiTrap Q XL, 1 ml	0.5	1	1	5	2	20	5
17-5159-01	HiTrap Q XL, 5 ml	0.5	5	5	25	10	100	25
17-1151-01	HiTrap SP HP, 1 ml	0.5	1	1	5	2	20	5
17-1152-01	HiTrap SP HP, 5 ml	0.5	5	5	25	10	100	25
17-5054-01	HiTrap SP FF, 1 ml	0.5	1	1	5	2	20	5
17-5157-01	HiTrap SP FF, 5 ml	0.5	5	5	25	10	100	25
17-5056-01	HiTrap CM FF, 1 ml	0.5	1	1	5	2	20	5
17-5155-01	HiTrap CM FF, 5 ml	0.5	5	5	25	10	100	25
17-5160-01	HiTrap SP XL, 1 ml	0.5	1	1	5	2	20	5
17-5161-01	HiTrap SP XL, 5 ml	0.5	5	5	25	10	100	25
11-0013-02	HiTrap Capto Q, 1 ml	0.5	1	1	5	2	20	5
11-0013-03	HiTrap Capto Q, 5 ml	0.5	5	5	25	10	100	25
28-9078-09	HiTrap Capto ViralQ, 5 ml	0.5	5	5	25	10	100	25
11-0032-73	HiTrap Capto MMC, 1 ml	0.5	1	1	5	2	20	5
11-0032-75	HiTrap Capto MMC, 5 ml	0.5	5	5	25	10	100	25
17-5441-22	HiTrap Capto S, 1 ml	0.5	1	1	5	2	20	5
17-5441-23	HiTrap Capto S, 5 ml	0.5	5	5	25	10	100	25
28-4058-44	HiTrap Capto adhere, 1 ml	0.5	1	1	5	2	20	5
28-4058-46	HiTrap Capto adhere, 5 ml	0.5	5	5	25	10	100	25
28-9165-37	HiTrap Capto DEAE, 1 ml	0.5	1	1	5	2	20	5
28-9165-40	HiTrap Capto DEAE, 5 ml	0.5	5	5	25	10	100	25
17-5190-01	HiPrep 16/10 Q FF	0.5	5	5	100	40	400	100
17-5192-01	HiPrep 16/10 SP FF	0.5	5	5	100	40	400	100

The table continues on next page.

Code No.	Column vol. ml	Pressure*	Flow MPa	Frac.vol ml/min	Equil.vol ml	Wash1 vol. ml	Elu.vol ml	Wash2 ml
17-5092-01	HiPrep 16/10 Q XL	0.5	5	5	100	40	400	100
17-5093-01	HiPrep 16/10 SP XL	0.5	5	5	100	40	400	100
17-5091-01	HiPrep 16/10 CM FF	0.5	5	5	100	40	400	100
17-5090-01	HiPrep 16/10 DEAE FF	0.5	5	5	100	40	400	100
17-1064-01	HiLoad™ 16/10 Q Sepharose HP	0.5	3	5	100	40	400	100
17-1137-01	HiLoad 16/10 SP Sepharose HP	0.5	3	5	100	40	400	100
Hydrophobic interaction								
17-1355-01	HiTrap Phenyl FF (high sub), 1 ml	0.5	1	1	5	2	20	5
17-5193-01	HiTrap Phenyl FF (high sub), 5 ml	0.5	5	5	25	10	100	25
17-1353-01	HiTrap Phenyl FF (low sub), 1 ml	0.5	1	1	5	2	20	5
17-5194-01	HiTrap Phenyl FF (low sub), 5 ml	0.5	5	5	25	10	100	25
17-1351-01	HiTrap Phenyl HP, 1 ml	0.5	1	1	5	2	20	5
17-5195-01	HiTrap Phenyl HP, 5 ml	0.5	5	5	25	10	100	25
17-1359-01	HiTrap Octyl FF, 1 ml	0.5	1	1	5	2	20	5
17-5196-01	HiTrap Octyl FF, 5 ml	0.5	5	5	25	10	100	25
17-1357-01	HiTrap Butyl FF, 1 ml	0.5	1	1	5	2	20	5
17-5197-01	HiTrap Butyl FF, 5 ml	0.5	5	5	25	10	100	25
17-0978-13	HiTrap Butyl-S FF, 1 ml	0.5	1	1	5	2	20	5
17-0978-14	HiTrap Butyl-S FF, 5 ml	0.5	5	5	25	10	100	25
28-4110-01	HiTrap Butyl HP, 1 ml	0.5	1	1	5	2	20	5
28-4110-05	HiTrap Butyl HP, 5 ml	0.5	5	5	25	10	100	25
17-5095-01	HiPrep 16/10 Phenyl FF (high sub)	0.5	5	5	100	40	400	100
17-5094-01	HiPrep 16/10 Phenyl FF (low sub)	0.5	5	5	100	40	400	100
17-5097-01	HiPrep 16/10 Octyl FF	0.5	5	5	100	40	400	100
17-5096-01	HiPrep 16/10 Butyl FF	0.5	5	5	100	40	400	100
17-1085-01	HiLoad 16/10 Phenyl Sepharose HP	0.5	3	5	100	40	400	100
Gel filtration								
17-1165-01	HiPrep 16/60 Sephacryl™ S-100	0.5	0.5	5	250	0	180	–
17-1194-01	HiPrep 26/60 Sephacryl S-100	0.5	1	6	250	0	480	–
17-1166-01	HiPrep 16/60 Sephacryl S-200	0.5	0.5	5	250	0	180	–
17-1195-01	HiPrep 26/60 Sephacryl S-200	0.5	1	6	650	0	480	–
17-1167-01	HiPrep 16/60 Sephacryl S-300	0.5	0.5	5	250	0	180	–
17-1196-01	HiPrep 26/60 Sephacryl S-300	0.5	1	6	650	0	480	–
17-1139-01	HiLoad 16/60 Superdex™ 30 pg	0.5	0.5	5	250	0	180	–
17-1140-01	HiLoad 26/60 Superdex 30 pg	0.5	1	6	250	0	480	–
17-1068-01	HiLoad 16/60 Superdex 75 pg	0.5	0.5	5	250	0	180	–
17-1070-01	HiLoad 26/60 Superdex 75 pg	0.5	1	6	250	0	480	–
17-1069-01	HiLoad 16/60 Superdex 200 pg	0.5	0.5	5	250	0	180	–
17-1071-01	HiLoad 26/60 Superdex 200 pg	0.5	1	6	250	0	480	–

* The pressure limits 0.35 and 0.5 MPa, respectively includes the pressure limit of the column (0.15 and 0.3 MPa, respectively) and the backpressure that the flow restrictor after the column creates (0.2 MPa).

Note: Don't forget to check that you have a sufficient number of tubes for fractionation. The templates are pre-set to fractionate during the elution and wash2 phases.

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