Strep(II)-tagged protein purification
ÄKTAprime Plus

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.

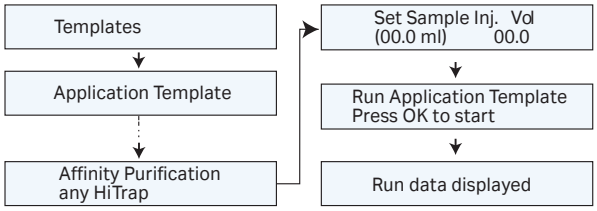
Preparing the sample

Step	Action
1	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.
2	Pass the sample through a 0.45 µm filter.

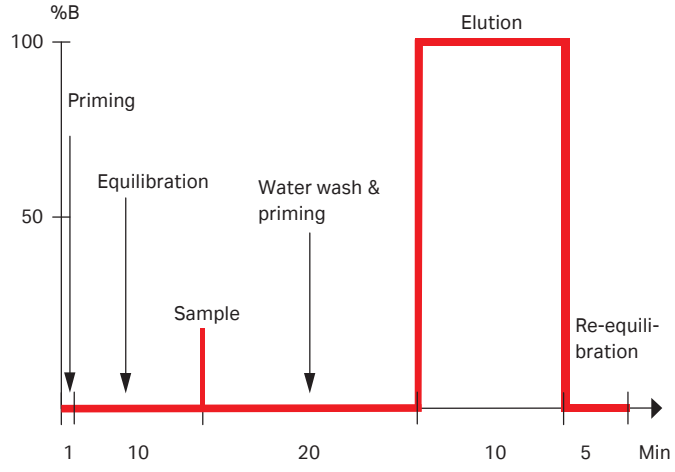
Preparing the system

Step	Action
1	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.
2	Place the three brown waste tubings in waste.
3	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).
4	Fill the fraction collector rack with 18 mm tubes[1] (minimum 10) and position the white plate on the fractionation arm against the first tube.
5	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.*

Selecting Application Template and starting the method

Step	Action
1	Check the communication to PrimeView™. At the lower right corner of the screen the text ***Controlled By →prime*** should be displayed.
2	Use the arrow and ***OK*** buttons to move in the menu tree until you find ***Affinity Purification any HiTrap***. 
3	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.*

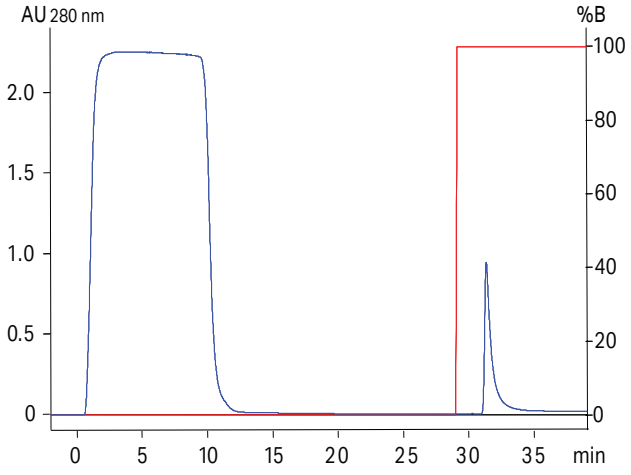


Total separation time = 47 min + sample application time

[1] 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.

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	Product code
StrepTrap HP	5 × 1 mL	28907546
HiTrap Desalting	5 × 5 mL	17140801
	100 × 5 mL[1]	11000329
HiPrep 26/10 Desalting	1 (53 mL)	17508701
	4 (53 mL)	17508702
Superloop 10 mL	1	18111383
Superloop 50 mL	1	18111384
Superloop 150 mL	1	18102385

[1] Pack size available by special order

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28928469 AC V:4 03/2021