On-Column Refolding

ÄKTA™prime with HiTrap™

1 Selecting Application Template
By using the arrow and OK buttons you move in the menu tree until you find the template you want to use.

2 Sample preparation
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 Buffer preparation
To ensure the best results use high purity water and chemicals. It is recommended to filter buffers by passing them 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

Wash eluent (port A2): Distilled water

Ni-loading eluent (port A3): 100 mM NiSO₄ in dist. H₂O

Solubilisation buffer (port A4): 6 M urea, 20 mM Tris-HCl, 0.5 M NaCl, 5 mM imidazole, 1 mM 2-mercaptoethanol, pH 8.0

Elution buffer (port A5): 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

4 Purification set-up
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. Place the three brown waste tubings in waste.
b) Connect the column between port 1 on the injection valve (7 port valve) and the UV flow cell.
c) Fill the fraction collector rack with 18 mm tubes (minimum 40) and position the white plate on fractionation arm against the first tube.
d) 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. If a Superloop™ is needed additional information is supplied in the instructions for the Superloop.
e) Set the range of the two channels on the recorder to 1 V and the speed to 5 mm/min.

5 Start method
• Check that the recorder is set up according to manual and ready for operation.
• Enter the sample volume and press OK to start the template.
**Trouble shooting**

**High back pressure:**
- 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 back pressure > 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.

**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.

### Ordering information

<table>
<thead>
<tr>
<th>Product</th>
<th>Quantity</th>
<th>Code No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>HiTrap Chelating HP</td>
<td>5 x 1 ml</td>
<td>17-0408-01</td>
</tr>
<tr>
<td></td>
<td>1 x 5 ml</td>
<td>17-0409-01</td>
</tr>
<tr>
<td>HisTrap™ Kit</td>
<td>1 kit</td>
<td>17-1880-01</td>
</tr>
<tr>
<td>HiTrap Desalting</td>
<td>5 x 5 ml</td>
<td>17-1408-01</td>
</tr>
<tr>
<td>HiPrep 26/10 Desalting</td>
<td>1 (53 ml)</td>
<td>17-5087-01</td>
</tr>
<tr>
<td>Superloop 10 ml</td>
<td>1</td>
<td>18-1113-83</td>
</tr>
<tr>
<td>Superloop 50 ml</td>
<td>1</td>
<td>18-1113-84</td>
</tr>
<tr>
<td>Superloop 150 ml</td>
<td>1</td>
<td>18-1023-85</td>
</tr>
</tbody>
</table>

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**to order:**

Asia Pacific: Tel: +852 2811 9693 Fax: +852 2811 5251 Australasia: Tel: +61 2 9899 0999 Fax: +61 2 9899 7511 Australia: Tel: 01 576 0616 20 Fax: 01 576 0616 27 Belgium: Tel: 0800 73 888 Fax: 03 272 1637 Canada: Tel: 1 800 463 5800 Fax: 1 800 567 1008 Central, East, South East Europe: Tel: +43 1 982 3826 Fax: +43 1 985 8327 Denmark: Tel: 45 16 2400 Fax: 45 16 2424 Finland & Baltics: Tel: +358 (0)9 512 3940 Fax: +358 (0)9 512 1710 France: Tel: 01 69 35 67 00 Fax: 01 69 41 96 77 Germany: Tel: 0761 4903 401 Fax: 0761 4903 405 Italy: Tel: 02 2732 21 Fax: 02 2732 212 Japan: Tel: 01 3 5331 9336 Fax: +81 3 5331 9370 Latin America: Tel: +55 11 3933 7300 Fax: +55 11 3932 7315 Middle East and Africa: Tel: +20 (1) 96 06 687 Fax: +20 (1) 96 09 693 Netherlands: Tel: 0165 580 410 Fax: 0165 580 401 Norway: Tel: 21 417 7035 Fax: 21 417 3184 Russia & other C.I.S. & N.I.S.: Tel: +7 (095) 232 0250 Fax: +7 (095) 230 6377 South East Asia: Tel: 60 3 8024 2080 Spain: Tel: 93 594 49 50 Fax: 93 594 49 55 Sweden: Tel: +46 18 612 19 00 Fax: +46 18 612 19 10 Switzerland: Tel: 01 802 81 50 Fax: 01 802 81 51 UK: Tel: 0800 616 928 Fax: 0800 616 927 USA: Tel: +1 800 526 3593 Fax: +1 877 295 8102

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