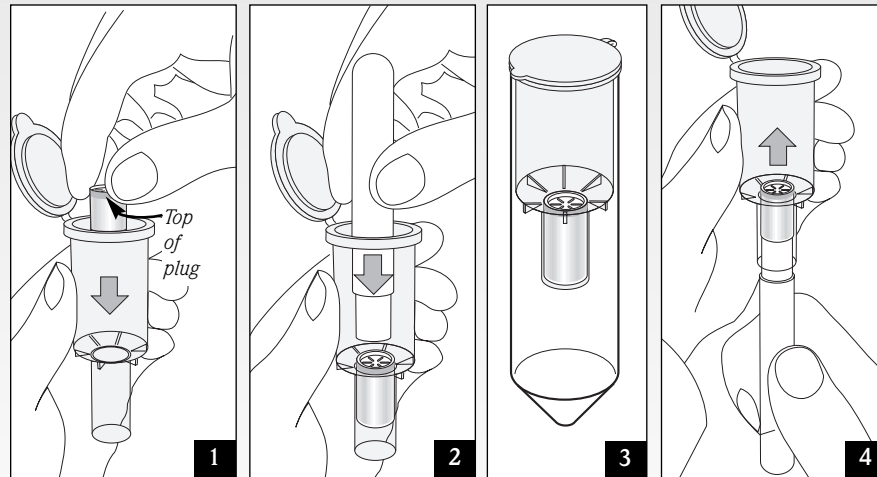
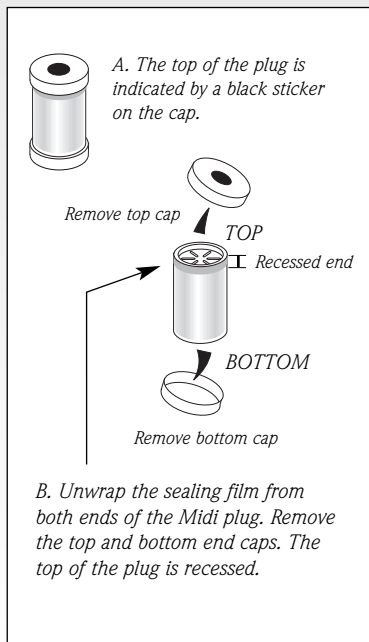


# MIDI

## Loading and removing the plug from the spin column



Place the plug into the spin column with the recessed end uppermost. Push the plug **FULLY** into the tapered end of the spin column using the plug insertion tool. It is now ready for pre-equilibration with binding buffer followed by centrifugation. After use, the plug is removed using the plug insertion tool.

IMAC

### Step by step protocol for Midi Spin Columns

#### RESIN PLUG LOADING

1. Load the pre-packed resin Midi plug containing immobilized IMAC resin into the Proteus spin column barrel using the insertion tool.

#### PRE-EQUILIBRATION (Total spin time = 3 mins)

2. Equilibrate the IMAC spin column with 10 ml binding buffer, pH 7.4 (10 mM imidazole) by centrifuging the spin column at 500 g for 3 min.

\* If 1 spin column is to be used, ensure that the spin column is counterbalanced in the centrifuge with a 50 ml centrifuge tube filled with the correct level of water.

#### CLARIFICATION OF SAMPLE

3. Filter 22-25 ml sample first through a 1.2  $\mu$ m pore size syringe filter and then immediately afterwards through a 0.2  $\mu$ m pore size syringe filter to remove any cellular debris, precipitating protein complexes just prior to sample loading.

#### SAMPLE LOADING (Total spin time = 30 mins)

4. Pipette up to 20 ml filtered cleared lysate into the spin column and centrifuge the spin column at 100-150 g for 30 min. It may be necessary to increase the spin speed or spin time if any sample remains above the plug.

N.B. In some circumstances, you may wish to re-apply the sample wash back through the spin column before the wash step in order to increase the residence time between the target protein and the resin plug for efficient binding.

### Recommended Buffers\*

**Binding Buffer:** 50 mM sodium phosphate buffer, 300 mM NaCl, 10 mM imidazole pH 7.4

**Wash Buffer:** 50 mM sodium phosphate buffer, 300 mM NaCl, 30 mM imidazole pH 7.4

**Elution Buffer:** 50 mM sodium phosphate buffer, 300 mM NaCl, 300 mM imidazole pH 7.4

\*See Table 1 on page 23 of IMAC handbook showing how to prepare working buffers of stock solutions supplied in the kit.

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**WASHING (Total spin time = 9 mins)**

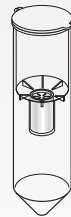
5. Wash the spin columns up to three times with 10 ml wash buffer pH 7.4 (30 mM imidazole) to remove non-tagged proteins with no affinity for the immobilized metal ion by centrifuging the spin columns for 3 min at 500 g. The washes should be collected for analysis. As imidazole absorbs UV radiation at 280 nm, we recommend that the wash buffer is used as the reference solution for auto-zeroing the UV-Vis spectrophotometer if imidazole is used to elute the target protein from the spin columns.

**ELUTION (Total spin times = 6 mins)**

6. Elute the bound His-tagged protein with 10 ml elution buffer pH 7.4 (300 mM imidazole) directly into a fresh centrifuge tube by centrifuging the spin columns for 3 min at 500 g. The eluate should be collected for further analysis. Repeat the above elution procedure faithfully to ensure complete recovery of all recombinant proteins.

N.B. Check the protein content of each eluted fraction before pooling them. Otherwise, you risk diluting a concentrated, purified sample.

**Pure Protein**



Used Spin Column

**DESALTING AND CONCENTRATING**

7. Imidazole and any residual metal ions should be removed by diafiltration using ultrafiltration concentrators or rapid dialysis against an appropriate buffer for your downstream application. Otherwise, the imidazole may strip the metal ion from a metalloprotein of interest or the target protein may irreversibly precipitate out of solution when stored at  $-20^{\circ}\text{C}$  or  $-80^{\circ}\text{C}$ .

**REGENERATION OF THE IMAC MIDI PLUG**

8. Wash the Midi plugs with 10 ml elution buffer by centrifuging the spin columns at 500 g for 3 min. Then wash the plugs with 10 ml binding buffer by centrifuging the spin columns at 500 g for 3 min. Proceed to the pre-equilibration step of another bind-wash-elute cycle if the plugs are to be re-used immediately. After regeneration, plugs can also be stored, without their end caps, in 0.1 % sodium azide (made up in distilled water) at  $2-8^{\circ}\text{C}$  until further use.

**PROTEUS**

**Easy-to-read Midi Purification Protocol:**

<i><b>Fraction</b></i>	<i><b>Volume</b></i>	<i><b>Step</b></i>	<i><b>RCF</b></i>	<i><b>Spin time</b></i>
Pre-equilibration	10 ml	Binding buffer	500 g	3 min
Sample load	Up to 20 ml	0.2 $\mu$ m filtered sample	100-150 g	30 min
Wash #1	10 ml	Wash buffer	500 g	3 min
Wash #2	10 ml	Wash buffer	500 g	3 min
Wash #3	10 ml	Wash buffer	500 g	3 min
Final Eluate #1	10 ml	Elution buffer	500 g	3 min
Final Eluate #2	10 ml	Elution buffer	500 g	3 min

**Easy-to-read Midi Regeneration Protocol:**

<i><b>Fraction</b></i>	<i><b>Volume</b></i>	<i><b>Step</b></i>	<i><b>RCF</b></i>	<i><b>Spin time</b></i>
Clean-up	10 ml	Elution buffer	500 g	3 min
Wash	10 ml	Binding buffer	500 g	3 min

**prochem**  
 ADVANCED PROTEIN SEPARATIONS

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