

## **Cleaning-in-Place Protocol**

We recommend that these Proteus spin columns are re-used with the same antibodies. However, if you want to re-use a Proteus spin column with a different antibody or you've experienced significant fouling of the plug after loading samples containing hydrophobic proteins, precipitated protein complexes, aggregated proteins, lipids or lipoproteins e.g. hyper-lipaedemic serum, bilirubinaemic serum or haemolysed and hyper-proteinaemic serum, use the following cleaning-in-place procedures; spinning the devices at 500 - 1,000 g for all steps. The following clean-up procedures do not result in a lower purification efficiency. However, please note that the most suitable cleaning procedure tends to be determined empirically and it does depend largely upon the nature of the previous sample loaded on to the spin column.

### **Cleaning-in-Place Procedure 1:**

- (i) Load at least 20 ml 4 M Urea (in water) or 6 M guanidine HCl (in water) on to the spin column. Remove the plug from the spin column barrel and incubate the plug for 1 hour at room temperature (RT) in a beaker containing 4 M urea or 6 M guanidine HCl.
- (ii) Re-insert the plug into the spin column barrel. Wash the column with a minimum of 20 ml distilled water.
- (iii) Wash the column further with at least 20 ml 0.3 % v/v HCl pH 1.5. Remove the plug from the spin column barrel and incubate the plug for 1 hour at RT in a beaker containing 0.3 % HCl pH 1.5.
- (iv) Immediately wash the column with at least 20 ml distilled water.
- (v) Then re-equilibrate the plug with a minimum of 10 ml binding buffer or PBS containing a suitable preservative e.g. 0.1 % NaN<sub>3</sub>, Proclin, 1% benzyl alcohol etc. Remove the plug from the spin column barrel and store it in a beaker containing binding buffer or PBS containing a suitable preservative at 4 °C.

**N.B. Always use fresh 6 M guanidine HCl. Never store the Protein A or G plugs in denaturants such as urea or guanidine HCl.**

### **Cleaning-in-Place Procedure 2:**

- (i) Wash the plug with a minimum of 10 ml non-ionic detergent (e.g. 0.1 % IGEPAL CA-630 or 0.1 % Triton X-100). Remove the plug and place it in a beaker containing 0.1 % of the same non-ionic detergent at 37 °C for 1 minute.
- (ii) Re-insert the plug into the spin column barrel and immediately wash the column with at least 2 x 20 ml distilled water.
- (iii) Then re-equilibrate the plug with a minimum of 20 ml binding buffer or PBS containing a suitable preservative e.g. 0.1 % NaN<sub>3</sub>, Proclin, 1% benzyl alcohol etc. Remove the plug from the spin column barrel and store it in a beaker containing binding buffer or PBS containing a suitable preservative at 4 °C.

### **Cleaning-in-Place Procedure 3:**

- (i) Wash the plug with at least 20 ml 70 % ethanol. Remove the plug from the spin column barrel and store it at room temperature in a beaker containing 70 % ethanol for 10-15 hours.
- (ii) Re-insert the plug into the spin column barrel and immediately wash the column with a minimum of 2 x 20 ml distilled water.
- (iii) Re-equilibrate the plug with at least 10 ml binding buffer or PBS containing a suitable preservative e.g. 0.1 % NaN<sub>3</sub>, Proclin, 1% benzyl alcohol etc. Remove the plug from the spin column barrel and store it in a beaker containing binding buffer or PBS containing a suitable preservative at 4 °C.