

## Application protocol for the usage of EndoTrap® with a HPLC / FPLC-automated system

### HPLC / FPLC column preparation

- Rinse the empty HPLC/FPLC column thoroughly with endotoxin-free water. Store over night in 1M NaOH to ensure no contamination with endotoxins. Afterwards wash the column with endotoxin-free water to remove residuals of NaOH completely.

### Preparation of HPLC / FPLC system

- Rinse the whole HPLC / FPLC system thoroughly with endotoxin-free water and 1 M NaOH. Keep in 1M NaOH over night to ensure no contamination with endotoxins. Afterwards wash the system with endotoxin-free water to remove residuals of NaOH completely.

### Preparation of EndoTrap column

1. Assemble the bottom part of the HPLC/FPLC column as described in the manufacturers' manual.
  2. Leave the columns' top open. Rinse with endotoxin-free water. Seal the outlet at the bottom after rinsing.
  3. Resuspend the EndoTrap slurry, so that no sediment is left.
  4. Fill the column with EndoTrap slurry. For this you can use a endotoxin-free funnel.
  5. Make sure that the column is closed at the bottom, to prevent the buffer from running out.
  6. Cover the top of the column with endotoxin-free Parafilm® or aluminium foil. For example use aluminium foil that has been heated to 200°C for 3h.
  7. When the resin is settled, open the outlet to drain the buffer, but do not let the resin run dry. Do not drain the buffer before the resin is completely settled, because air bubbles may form in the resin.
  8. Repeat the filling steps until all of the resin is in the column.
- Assemble the top part of the HPLC/FPLC column following the manufactures' manual.
  - The column can be used following the EndoTrap protocol (see below).

### Column Activation and Endotoxin Removal

1. Regenerate the column with 6x column volumes regeneration buffer (RB)\*.
2. Wash the column with 6x column volumes equilibration buffer (EB).
3. Apply sample onto the column and start collecting immediately. Applied protein elutes directly after the column void volume. Be care, that the run time is not be higher than 0.2-1 ml/min (max. pressure: 3 bar, 43 psi, 0.3 MPa) so that the gel bed does not compress. The slower the speed, the more efficient the endotoxin removal is. Afterwards elute the sample completely from column.

### Column Regeneration and Storage

1. Wash the column with 6x column volumes equilibration buffer (EB).
2. If you want to store the column, apply 6x column volumes of regeneration buffer (RB) supplemented **with 0.02% sodium azide**, remove & close the column and store at 4°C.  
OR:  
If you want to regenerate the column, make sure you start with **step 1** of "Column Activation and Endotoxin removal".

\* The regeneration substance is NOT (sodium) deoxycholate! The regeneration buffer RB (blue or red) can be ordered also separately (1x or 10x concentrated). Please inquiry.