Protocol

TD-P Revision 1.0

Creation Date: 10/8/2021
Revision Date: 7/20/2022

FPLC Column Packing Protocol

Introduction

GoldBio's FPLC column is suitable for both FPLC and ÄKTA design™ chromatography systems. The columns are disposable, easy to pack, identically designed across all sizes, with a robust construction allowing for moderate back pressure and are easy to store with long shelf life. Additionally, the FPLC column parts are made of polypropylene, which has been shown to have excellent chemical resistance to most reagents.

A resin-packed FPLC column can be readily stored and reused many times. GoldBio columns have standard connections, compatible with common chromatography instruments (such as ÄKTA). Additional upper frits are also included, however only one should be used in the column packing at a time.

Materials

- F-300-8x3 (8 ml):
  - 3 columns
  - 9 frits
  - 6 caps
- F-300-30x2 (30 ml):
  - 2 columns
  - 6 frits
  - 4 caps
- F-300-45x2 (45 ml):
  - 2 columns
  - 6 frits
  - 4 caps
- F-300-80x1 (80 ml):
  - 1 column
  - 3 frits
  - 2 caps

Method

1. Push the bottom frit firmly to the bottom of the column, remove the bottom cap and wet the frit by adding several ml of distilled water. Make sure to catch the wetting water solution with a syringe or similar beaker/container. Replace the lower cap.

2. It is recommended to increase the concentration of the resin bead suspension by removing most of the liquid supernatant of the standing resin solution before adding to the column. This can best be done by pipetting off the clear supernatant liquid from the bottle of resin where the beads are completely settled to the bottom and discarding. Leave enough liquid still allow easy pipetting transfer to the column.

3. Remove the lower cap of the FPLC column and slowly run the concentrated bead suspension down the walls of the FPLC column. Allow to stand for a while until the beads settle down completely. Add more of the bead suspension and repeat until the packed volume reaches the top of the column. Be careful not to dry the bed. Catch the expressed supernatant liquid as in 1.above.

   Note: it is advisable to add the agarose bead suspension slowly to avoid the formation of bubbles. The resin may also be degassed prior to adding to the column.

4. Wet one upper frit with distilled water and insert it on top of the filled resin column, taking care not to trap air. Screw the upper end plug onto the column* carefully. Connect inlet of the FPLC column to the device adaptor connector and set at the working flow rate for about 5 minutes.

   Note: Make sure no air is trapped under the top frit.

* The 80 ml FPLC column contains an additional diffusion plate that fits on top of the top frit. Our smaller FPLC columns (8 ml, 30 ml and 45 ml) do not have a diffusion plate.

Associated Products

- Empty FPLC Columns (GoldBio Catalog # F-300)
- Cobalt HTC Agarose Beads (GoldBio Catalog # R-203)
- Nickel HTC Agarose Beads (GoldBio Catalog # R-202)
- 6% HTC Agarose Beads, Standard (GoldBio Catalog # R-201)
- 4% HTC Agarose Beads, Standard (GoldBio Catalog # R-200)
- Protein G HTC Agarose Beads (GoldBio Catalog # P-430)
- Glyoxal HTC 6% High Density Agarose Beads (GoldBio Catalog # G-312)
- Nickel NTA HTC Agarose Beads (GoldBio Catalog # H-355)