

Pre-column User's Manual

A pre-column is applied to the system to avoid the contamination of a separation column by protein etc. The lifetime of a separation column depends on a state of a pre-column. Generally speaking, a pre-column can be used for approximately 100 times injection of 10 µl with blood or tissue denatured protein samples or for months with microdialysis samples. These lifetime can change dramatically with the way of denaturing of protein as sample preparation or pre-column condition. To identify the quality of the precolumn, please replace the precolumn with a two way joint and run standard sample only with a separation column. If the peak shape becomes good, the main column is in a good condition. If your samples are microdialysis samples, please replace the precolumn gel and in let filter at least once for three month.

Filter clogging of a pre-column raises outlet pressure of mobile phase pump. If this pressure rises more than 1.5 MPa than usual, an up stream filter must be exchanged and the pre-column must be repacked. Repack of a pre-column requires Prepak set. Select pre-column filler according to Eicom Application Manual.

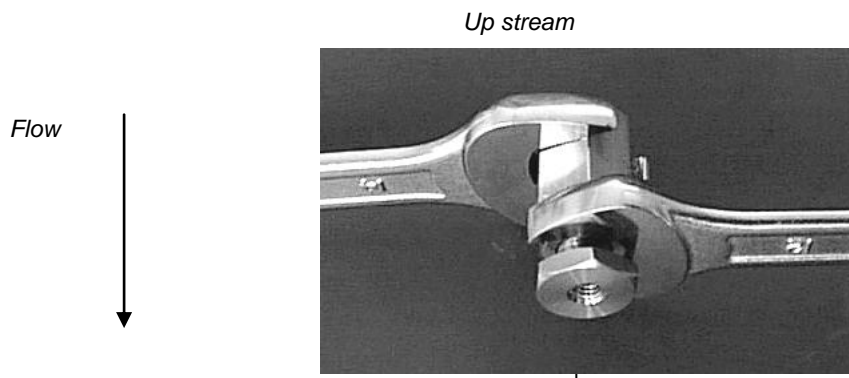
Selection of Pre-column Gel

Separation column	Pre-column packing gel
Columns for microdialysis	Any filler can be applied. (AC-ODS is the cheapest.) Set it up stream of an injector.
AC-GEL	CH-GEL
CA-5ODS	CA-ODS
GU-GEL	CH-GEL
SC-3ODS, SC-5ODS	AC-ODS CA-ODS (in case mobile phase pH is around 6)

If the standard flow rate is less than 300 µL, please use 3 mm ID precolumn (PC-03). If the flow rate is 300 µL ore more or the precolumn is set before injectors, please use 4 mm ID precolumn (PC-04).

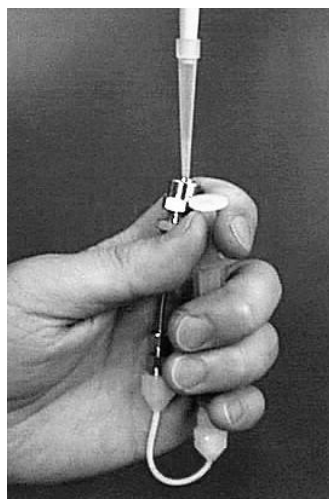
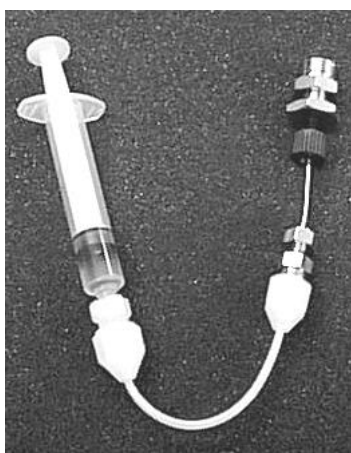
How to repack pre-columns.

Disconnect a pre-column take old gel in side of the column with using two wrenches. Then wash inside of the empty column with methanol.



Prepare slurry of packing gel: pure methanol in to the original bottle of the packing gel and put a cap back on. Then shake well. The gel never dissolves. The required methanol volume is about 30 mL for new bottle not necessary to be exact volume. Please adjust methanol volume based on the left packing gel amount in the bottle. The methanol will evaporate, even if the cap is tightly closed.

Please cut just small part of the tip of the pipette tip, size of 20~200 μ L, to make the aspiration of slurry easy with wider mouth. Set the pipette volume at 20 μ L. Set a syringe union and disposable syringe filled with 0.5 ml methanol as below. Fill up a pre-column with the slurry of packing gel with using a pipette. Then slowly aspirate methanol from a pre-column by a disposable syringe.

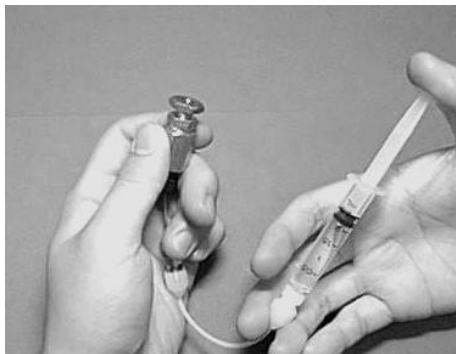


Repeat these steps until a pre-column is totally filled with the gel. The precolumn has to be filled completely in the precolumn and is better to be slightly over filled. Then tighten the two parts by wrench. The filter can easily fell off from the precolumn. Please pay caution to avoid it.

Important

Aspirate slowly methanol by the syringe and fill next slurry before the methanol completely dry out in the precolumn. Keep always a little bit methanol (methanol) in the precolumn while filling gel.

Remove the syringe connection parts. Take clean new methanol in the syringe and connect the syringe to the inlet of the precolumn. Then infuse methanol from the syringe to rinse inside. Rinse with methanol twice. Then rinse the precolumn with ultrapure water in the same way as above. Ultrapure water will get a lot of resistance in the precolumn. Please hold the connection part to the syringe.



After these steps connect the pre-column to the HPLC system (normally before the column after injectors, before injector in the case of microdialysis samples). First connect tube only to the up stream side and wash inside by pumping mobile phase. After several minutes connect tube to the down stream side too. Except for microdialysis, failure of pack of pre-column makes shapes of peaks strange.

If the peak shape of the standard sample with a repacked pre-column doesn't become better but the peak shape is good without a pre-column, the repacked pre-column is still not good. Try to repack the pre-column again. Most of the reason is the filled gel contains a gap. Please fill the gel slowly. If the specified gel is not packed, then the shape of peak is asymmetric.

Filter Exchange

When Methanol inside a pre-column is not aspirated smoothly by a syringe, the upstream filter (PF-04 for 4 mm precolumn and PF-03 for 3 mm precolumn) is clogged and this should be exchanged. The filter can be disconnected from a pre-column with a filter exchange tool (FP-05, see below).

