SepFast 26/10 1000-6000Da Desalting Column

26 x 100 mm

General Information

Column body (i.e. the tube): made of acrylic. It has an appearance similar to a glass tube, i.e. clear and transparent. This material is compatible to most commonly used aqueous chemicals. ***WARNING: It isn't compatible with concentrated alcohols. 20% ethanol can be used for storage purpose. Don't use any alcohols greater than 20% v/v.***

End plunger: made of POM with polyamide support mesh (15 μm). Its o-ring is of NBR. They are inert to most aqueous buffers.

Connection: 1/16" female thread in both sides.

End Cover: Made of POM

Hardware pressure: recommended rating is less than **6 bar** (or 0.6 MPa, or 84 psi).

Flowrate: recommend max. 40 ml/min

Storage: After receiving the columns, store them in a cold room.

Instruction of Use

Each packed column is sealed with pressured syringe in the bottom end of the column. It is then wrapped to a foam pad support.

1. Carefully unwrap the thin film to take out the column.
2. Follow the flow direction to clamp the column to a vertical stand.
3. Unscrew the top Stop Plug. Connect the column top to a chromatography system. Make sure that no air bubble is trapped in.
4. Gently unhook the springs from the shaft of the syringe using balanced force.
5. Twist with push-up force to unscrew the male-thread adaptor from the column outlet. Keep this storage syringe for later use.
6. Connect the outlet to the chromatography system.
7. Run at reduced flowrates (e.g. 10 ml/min) to wash away the storage solution then run at normal flowrate until the column is equilibrated.

CAUTION: Regularly check the end covers at both ends of the column during usage of the column. Make sure the end cover isn't loose. Screwing the cover tightly by two fingers (i.e. ***FINGER TIGHT***) if it becomes loose. ***NEVER SCREW THE COVER BY A FULL HAND***. It would damage the internal structure.

Storage after Use

In short term, seal both ends of the column with stop plugs if it isn't used.

In longer term, i.e. if the column isn't used for a few days or over, follow the instructions below to store the column.

1. Fill the column with 20% ethanol at 10 ml/min until the column is fully filled, e.g. 2CV.
2. Stop the pump. Screw a stop plug to seal the bottom side of the column.
3. Dis-connect the column top from the chromatography system.
4. Stop the pump.
5. Suck the storage solution to fully fill the storage syringe (including the male-thread and the tubing part). Leave the syringe upside-down and push out the air bubble. Adjust the liquid level inside the syringe to 4 ml.
6. Carefully screw the male thread part of the syringe system to the column top. Finger tight is enough.
7. Use balanced force to hook the springs back the top shaft of the syringe.
8. Check and make sure that there is no leak.
9. Place the column in a cold room.

Table 1. Key properties of the desalting medium

Bead structure	Dextran
Mean particle size	35 - 140 µm
Exclusion limit (MW)	5000 Dalton for globular protein
Chemical stability	All commonly used buffers
pH stability	
Long term	3 to 12
Short term	2 to 13
Storage	4ºC to 30ºC in 20% ethanol (or isopropanol) or 4ºC in 0.04% sodium hydroxide

Sample loading

Gel filtration is largely independent of sample concentration. The volume of the sample relative to the bed volume is far more important. For analytical purposes the sample should not be larger than 1-5% of the bed volume, whereas for desalting or buffer exchanging, the sample can be as large as 30-35% of the bed volume. The viscosity of the sample may limit the concentration of sample which can be used. Viscous samples may be diluted to decrease the viscosity. It may be possible to achieve better results by applying viscous samples at a lower flow rate. The sample should be clear, and completely dissolved in running buffer, without particles or solid contaminants. Filtration of samples will increase column life. If, due to the nature of the sample, it is not possible to filter it, the sample should be centrifuged until it is clear.

Void volume determination

The void volume (Vo) of the bed is equal to the elution volume (Ve) of excluded material. The void volume of the bed should be determined and the bed should be tested for uniformity of eluant flow before applying experimental sample. Coloured proteins such as haemoglobin or cytochrome C are convenient for this procedure.

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