

## Operational Instructions

### Disposable SepFast™ Column –16 mm i.d.

Glass columns are so expensive that people can't afford a large inventory. A user has to pack, unpack and repack the same column for different purification tasks. In particular, it could be very inconvenient if a laboratory needs to purify a large number of different molecules.

BioToolomics' range of disposable chromatography columns has all the functionalities of small glass columns but also possesses many other advantages, such as; low cost, flexibility, resin compressibility taken into account, disposable, easy to pack, tight packing of any type of chromatography media (>20 µm), identical design to the scaled-up columns, robust, precise and reproducible packing, allowing moderate back pressure, easy to store, long life time.

After the chosen resin is packed in the column, the column can be readily stored and used multiple times during the life time of the packed resin. Due to its low cost, a user can afford to assign individual columns for the purification of individual molecules.

### Column Construction



The **column body** (i.e. the tube) is made of acrylic. It has an appearance similar to a glass tube, i.e. clear and transparent.

The **End Plug** is made of polypropylene. In one end, it has the standard connection (10.32 UNF female thread) compatible with the common chromatography instruments (such as AKTA). In the other end, it has two layers of mesh (coarse and fine) giving excellent flow distribution. The void volume in each End Plug is minimal as the fluid is

introduced through a narrow flow path (i.e. 1 mm hole). Two O-rings are used in the End Plug to seal and secure the column. The O-rings are made of nitrile (Buna N) and the fine support mesh is made of polyamide.

The packed resin volume is 2 ml / cm bed height.

The column material shows excellent chemical resistance to most of the commonly used reagents (see chemical compatibility table below) . The recommended operational pressure is up to 3 bar (42 psi).



Chemical Compatibility Table

Chemical	Compatibility
Acetone	Poor - not recommended
Acetic Acid	Poor- not recommended
Ethanol	Poor - not recommended above 20% concentration
Guanidine Hydrochloride	Good
Hydrochloric Acid	Poor - not recommended for concentrated solutions
Isopropanol	Poor - not recommended
Methanol	Poor - not recommended above 20% concentration
Potassium Hydroxide	Good
Phosphoric Acid	Poor - not recommended
Sodium Hydroxide	Very Good below 80% concentration
Sulfuric Acid	Good below 10% concentration
Urea	Very Good

The following packing procedure works as a general guide. End-users may develop suitable packing protocols of their own media.

**Packing the column**

- The bottom End Plug has been fully inserted inside the column body on delivery.
- Carefully remove the top End Plug that is partially inserted in the column body. Avoid direct contact of the mesh with the column wall.
- Screw a 10-32 male thread / female luer connector (not supplied) to the End Plug. Keep the open side of the column upward and vertical. Hold the column by hand or to a stand.
- Use a syringe (20 ml or 50 ml syringe depending on the packed volume) pre-filled with 2 – 5 ml of water (buffer) to fill the column to a level of 1 – 2 ml. This step is to purge the air in the End Plug. Keep the syringe in place.
- Shake to mix the resin well. Pour or pipette the resin slurry in. Suck the bed down with the syringe. The level of the packed resin can be clearly seen. The bed permeability decreases with the increase of bed height. If the syringe is fully filled, discharge the liquid and re-connect the syringe to the End Plug. Be careful not to dry the bed during the packing process. Pour or pipette in more slurry or water (buffer), or pipette out more slurry until the packed volume (under suction) reaches the desired level. **Note: the compressibility depends on the type of resin. As a guide, the packed volume should be 10% to 25% more than the final target volume for agarose-based spherical particles.**
- Keep the syringe in place. Be sure that there is at least 5 ml space not filled in the syringe. Pipette water (buffer) in to top off the column. Carefully insert the top End Plug (to avoid trapping of air bubble). Push it down slowly until the double O-rings fully get into the column body. Screw a Stop Plug to seal the top. Push the End Plug down until it is fully engaged in the column body.
- Remove the 10-32 male thread / female luer connector and the syringe. Screw another Stop Plug into the bottom End Plug. The column is now ready for short-term storage.

- Depending on the nature of individual resins, this step may be operated to further settle the particles in the bed. Pre-fill a syringe with liquid (ideally the same liquid as the one in the column). Insert it to a 10-32 male/luer female connector and purge out any air in the flow path. Remove the top Stop Plug. Attach the pre-filled syringe to the top End Plug (be sure that no air is trapped in the flow path). Remove the bottom Stop Plug. Push through at least 5 bed volumes of liquid under pressure (e.g as fast as possible) by hand. Seal the bottom with a Stop Plug. Disconnect the syringe and then seal the top. This step can also be done by connecting the column to a chromatography system (such as AKTA). Pump through 10 bed volumes of equilibration buffer at a flowrate at least 30% higher than your operational flowrate. Be sure the back pressure is always under 3 bar.

**Note: after a column is properly packed and used, it can't be disassembled for another packing as the mesh in the End Plug will be destroyed.**

## Ordering information

Product*	Quantity**	Code no.
SepFast column– 20 ml	1 unit (1 column body + 2 End Plugs)	240112
SepFast column – 30 ml	1 unit (1 column body + 2 End Plugs)	240113
SepFast column – 40 ml	1 unit (1 column body + 2 End Plugs)	240114
Stop Plug	10 / pack	240104
10.32 male thread / female luer connector	5 / pack	240105

**\*For columns of other volumes or higher pressure ratings, please contact us for a quotation. BioToolomics can offer columns of other bed heights (from 0 cm to 60 cm) and higher pressure ratings.**

**\*\*The minimum order is 5 columns. If a large quantity is required, please contact us for further information. BioToolomics also provides services to pack customers' media of their choice.**

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