

Operational Instructions

Disposable SepFast™ Column (1 ml, 0.67 ml, 0.33 ml)

The current pre-packed columns in the market have poor scalability and poor mechanical strength. Due to the wall-support effect and the rigidity of individual chromatography resins, the particle has to be correctly compressed even after settling down under flow packing. It will then form a tightly packed bed with predictable chromatographic performance relatable to the scaling-up columns.

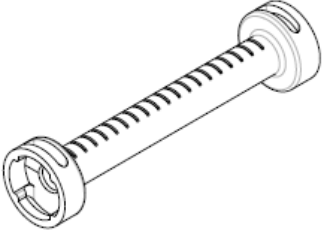
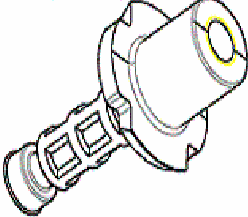
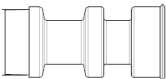
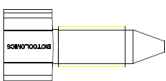
BioToolomics' range of disposable chromatography columns have all the functionalities of a small re-usable column but also possesses many other advantages, such as; accounted for resin compressibility, disposable, easy to pack, tight packing of any type of chromatography media (>20 µm), identical design to the scale-up columns, robust, precise and reproducible packing, allowing for moderate back pressure, easy to store and long life time.

After the chosen resin is packed in the column, the column can be readily stored and used multiple times.

The column parts are mostly made of polypropylene, a material which shows excellent chemical resistance to most of the commonly used reagents (details of chemical resistance can be found in the chemical compatibility table below). The columns have the standard connection which is compatible with common chromatography instruments (such as AKTA).

The recommended operational pressure is up to 5 bar, as most of the process chromatography media allows an operational pressure less than 3 bar.

Description of the Column Parts

Part	Description
	<p>Column Body: the internal diameter is 6.2 mm. The volumetric marks are at intervals of 0.1 ml. It contains a locking mechanism in both ends. Made of polypropylene.</p> <p>Quantity supplied: 1</p>
	<p>End Plug: it has 10-32 UNF female thread in one end and an O-ring and frit disc the other end. It produces the minimum void volume in the column. End plug body is made from polypropylene. O- ring is made from nitrile (Buna N). Frit is made from polyethylene.</p> <p>Quantity supplied: 2</p>
	<p>Extension Plug: it allows a volume change of the packed bed. It has an O-ring and frit disc in one end. Extension plug body is made from polypropylene. O- ring is made from nitrile (Buna N). Frit is made from polyethylene.</p> <p>Quantity supplied: 1 (for 0.67 ml) and 2 (for 0.33 ml)</p>
	<p>Stop Plug: it has 10-32 UNF male thread. It is used for the finger tight seal of the packed column. Made of polypropylene.</p> <p>Quantity supplied: order in separate</p>

Chemical Compatibility Table

Chemical	Compatibility
Acetone	Poor - not recommended
Acetic Acid	Good at 20% concentration or pure. Poor at 80% concentration
Ethanol	Good
Guanidine Hydrochloride	Very Good
Hydrochloric Acid	Good
Isopropanol	Very Good
Methanol	Excellent
Potassium Hydroxide	Very Good
Phosphoric acid	Poor - not recommended
Sodium Hydroxide	Very Good at below 80% concentration
Sulfuric Acid	Very Good at below 75% concentration
Urea	Very Good

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

Packing 1 ml column

- Insert one End Plug into one end of the Column Body. Push the End Plug until it is fully engaged with the shoulder of the Column Body.
- Screw a 10-32 male/luer female connector (not supplied) to the End Plug. Use a syringe pre-filled with 1 – 2 ml of water to fill the column to a level of 0.2 – 0.3 ml.
- Pipette the resin slurry in and suck the bed down with the syringe. Be careful not to dry the bed. If necessary, pipette in more water or slurry, or pipette out more slurry until the packed volume (under suction) reaches the desired level. **Note: the packed volume depends on the type of resin. As a guide, the packed volume should be 1.2 – 1.4 ml for agarose-based spherical particles.**
- Pipette water in to fully fill the column. Carefully insert the top End Plug (to avoid trapping air bubbles). Push it down slowly until the liquid level reaches the thread. Stop pushing and screw a stop plug to seal the top. Then push the top End Plug down until it is fully engaged in the locking mechanism. Note: don't remove the syringe in this stage.
- Remove the 10-32 male/luer female connector and the syringe. Screw another Stop Plug into the bottom End Plug.
- 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 10 ml liquid (ideally the same liquid as the one in the column). Insert it into 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 ml 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) to pump liquid through at high speed (e.g. 3-5 ml/min).

Packing 0.67 ml column

- Connect one End Plug to the Extension Plug. Insert the assembly into one end of the Column Body. Push the End Plug until it is fully engaged with the shoulder of the Column Body.
- Screw a 10-32 male/luer female connector (not supplied) to the End Plug. Use a syringe pre-filled with 1 – 2 ml of water to fill the column to a level of 0.2 – 0.3 ml.
- Pipette the resin slurry in and suck the bed down with the syringe. Be careful not to dry the bed. If necessary, pipette in more water or slurry, or pipette out more slurry until the packed volume (under suction) reaches the desired level. **Note: the packed volume depends on the type of resin. As a guide, the packed volume should be 0.8 – 0.9 ml for agarose-based spherical particles.**
- Pipette water in to fully fill the column. Carefully insert the other End Plug (to avoid trapping of air bubble). Push it down slowly until the liquid level reaches the thread. Stop pushing and screw a stop plug to seal the top. Then push the top End Plug down until it is fully engaged in the locking mechanism. Note: don't remove the syringe in this stage.
- Remove the 10-32 male/luer female connector and the syringe. Screw another Stop Plug into the bottom End Plug.
- 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 10 ml 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 ml 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) to pump liquid through at high speed (e.g. 3-5 ml/min).

Packing 0.33 ml column

- Connect each End Plug to each Extension Plug.
- Insert one of the above assemblies into one end of the Column Body. Push the End Plug until it is fully engaged with the shoulder of the Column Body.
- Screw a 10-32 male/luer female connector to the End Plug. Use a syringe pre-filled with 1 – 2 ml of water to fill the column to a level of 0.2 – 0.3 ml.
- Pipette the resin slurry in and suck the bed down with the syringe. Be careful not to dry the bed. If necessary, pipette in more water or more slurry or pipette out more slurry until the packed volume (under suction) reaches the desired level. **Note: the packed volume depends on the type of resin. As a guide, the packed volume should be 0.4 – 0.5 ml for agarose-based spherical particles.**
- Pipette water in to fully fill the column. Carefully insert the other assembly (to avoid trapping of air bubble). Push it down slowly until the liquid level reaches the thread. Stop pushing and screw a stop plug to seal the top. Then push the top End Plug down until it is fully engaged in the locking mechanism. Note: don't remove the syringe in this stage.
- Remove the 10-32 male/luer female connector and the syringe. Screw another Stop Plug into the bottom End Plug.
- 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 10 ml liquid (ideally the same liquid as the one in the column). Insert it into 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 3 ml 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) to pump liquid through at high speed (e.g. 3-5 ml/min).

Ordering information

Product	Quantity*	Code no.
SepFast column – 1 ml	1 unit (1 column body + 2 End Plugs)	240101
SepFast column – 0.67 ml	1 unit (1 column body + 2 End Plugs + 1 Extension Plug)	240102
SepFast column – 0.33 ml	1 unit (1 column body + 2 End Plugs + 2 Extension Plug)	240103
Stop Plug	10 / pack	240104
10.32 male thread / female luer connector	5 / pack	240105

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

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