Sanitization

After packing and between runs, sanitization and depyrogenation of the sorbent may be necessary. The following procedures are recommended:

Method	Procedure
Base treatment	Wash at 25°C (77°F) with 5 cv of 0.5 M NaOH. One (1) hour contact time with sorbent is recommended. Re-equilibrate column with sterile pyrogen-free buffer.
Alcohol/acid treatment	Wash with at least 3 cv of a solution of 20% (v/v) ethanol containing 1 M acetic acid. One (1) hour contact time with sorbent is recommended. Re-equilibrate column with sterile pyrogen-free buffer.

For more information, please contact our technical service.

Thermal stability and storage

Temperature of use	2-30°C (36-86°F)
Shipping temperature	Ambient
Storage temperature	2-30°C (36-86°F) (2-8°C / 36-46°F once opened)
Storage solution between runs	Neutral buffer containing bacteriostatic agents such as 20% (v/v) ethanol, 1 M NaCl and 1.2 mM EDTA.
Caution	Must never be frozen

Ordering information

Pack size	Part Number
25 mL	20051-033
100 mL	20051-025
1 L	20051-017
5 L	20051-041
10 L	20051-058

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BioSepra™ Methyl Ceramic HyperD® F

Methyl Ceramic HyperD® F

Hydrophobic Interaction Chromatography Sorbent

Product Description

Methyl Ceramic **HyperD** F high capacity sorbent is designed for efficient and scalable capture of biomolecules.

The sorbent employs a high-capacity hydrogel polymerized within the large pores of a rigid ceramic bead. This design combines the desirable characteristics of a soft, high capacity hydrogel with the high dimensional stability of a rigid ceramic bead filled with a hydrophobic (-CH_3) functionalized hydrogel.

Ceramic **HyperD** sorbent does not shrink or swell with changes in pH or conductivity. This unique structure provides a hydrophobic sorbent with superior dynamic capacity for proteins over a full range of linear velocities.

Properties

Average particle size (µm)	50
Capacity (mg/mL)(1)	>16
Functionalized groups (µeq/mL)	-CH_3
Working pH	2-12
Cleaning pH	1-14
Volume changes due to pH and ionic strength	Non compressible
Pressure resistance	70 bar (1,000 psi)

(1) Dynamic binding capacity determined at 10% breakthrough, 100 cm/h using sodium phosphate / 2 M ammonium sulfate, pH 7.

Column packing

• General consideration

Methyl Ceramic **HyperD** F sorbent is provided in ready-to-use lab-packs of 25 mL, 100 mL, and 1 L as well as in drums of 5 L and 10 L for process-scale applications. The sorbent is supplied as a slurry (70-75% v/v) in a buffer containing 1 M NaCl, 1.2 mM EDTA and 20% (v/v) ethanol.

• Preparing Sorbent for Column Packing

IMPORTANT: Use of buffers with a minimum ionic strength of 5 mS/cm is recommended throughout all processes using Methyl Ceramic **HyperD** sorbent.

1. Gently agitate the container in which the sorbent has been supplied. Resuspension by inversion or by stirring with a plastic paddle is recommended. Do not use magnetic stir bars at any point in the process.

2. Transfer a suitable volume of slurry to a graduated cylinder. Include a practical excess of sorbent (5-10%) of the desired bed volume. Allow the sorbent to settle for approximately 15 minutes and then remove the supernatant by decanting.
3. To the settled bed, add an equal volume of packing buffer. Gently resuspend the sorbent with a plastic paddle and transfer the slurry into a larger container.
4. Add 4-8 volumes of packing buffer to the slurry and gently stir to achieve a homogenous suspension. Allow the sorbent to settle for approximately 15 minutes and then remove the supernatant by decanting.
5. Repeat the last step 2-3 more times to fully equilibrate the sorbent and eliminate any fine particles.
6. After decanting the final supernatant, add a volume of packing buffer equal to half the settled sorbent to achieve a 66/33% sorbent/liquid volume ratio.

• Packing a small column (< 5 cm / 2 in. ID)

Ceramic **HyperD** F sorbent is compatible with traditional low or medium pressure chromatography columns and equipment. A column equipped with an adjustable flow adapter facilitates optimal packing and is recommended. For preliminary laboratory studies, a column of 1 cm (0.4 in.) ID x 15 cm (5.9 in.) is recommended. Scale-up is best accomplished according to well-known principles: the bed height is maintained constant while diameter is increased.

A Methyl Ceramic **HyperD** F column may be packed according to the required operational flow rate. Set up the column and introduce the lower adapter. Connect 1 m long tube to the adapter outlet. Fix a syringe to the outlet tubing and then introduce degassed buffer up to 2-10 mm high. Make sure that all air bubbles are thoroughly eliminated from the outlet if the column. Clamp the column outlet tubing and remove the syringe. If necessary, put a filling reservoir on the top of the column and then follow one of the methods described below.

Packing with hydrostatic pressure with an LPLC column

1. Pour the degassed suspension into the column. Allow it to settle for a few seconds.
2. Open the column outlet and set the hydrostatic pressure to a minimum of 80 cm (31 in.). Column packing is complete when the sorbent is totally sedimented. Never let the upper part of the column dry (add buffer if necessary).
3. Close the column outlet and dip the upper adapter a few millimeters into the sorbent
4. Clamp the outlet of the upper tubing after checking for air bubble elimination,
5. Connect a pump to the column outlet.

Packing with pump pressure

1. Pour the degassed suspension into the column.
2. Connect a peristaltic pump to the upper part of the column.
3. Open the column outlet and use a flow rate roughly 20-30% higher than that used for the separation.
4. Once the sorbent is well sedimented, disconnect the pump and adjust the upper adapter.

• Packing a large column (> 5 cm / 2 in. ID)

Please contact our technical service.

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Working conditions

• Column washing and equilibration

After packing the column, wash with 1.5 cv (column volume) of a buffer of the same composition as that chosen for equilibration but at a higher salt concentration. Follow the wash procedure with 3-5 cv of equilibration buffer. Continue to equilibrate the column until the ionic strength and pH of the buffer at both the outlet and the inlet of the column are identical.

• Sample application

Ensure the absence of bubbles in the sample. Inject the sample into the column through a pump and then connect the pump to a buffer reservoir or to a gradient system. Start simultaneously the pump, the recorder and the elution gradient maker.

• Working flow rate

As Methyl Ceramic **HyperD** F sorbent is rigid, columns that are prepared as recommended can be used at very high flow rates. During preliminary studies, it is recommended that flow rates of 100 cm/h first be used and then be increased to 300 cm/h and more.

• Choice of elution gradient and its slope

The elution mechanism of a hydrophobic sorbent is based on restoring solvation water (removing the salt) and/or the direct competition with the hydrophobic mechanism such as the addition of organic solvents.

We recommend the following procedure: the linear gradient should correspond to 10 cv starting from the binding buffer with a salt concentration of 150 mS/cm (i.e. 50 mM phosphate sodium / 2 M ammonium sulfate) to a buffer with a salt concentration of 5 mS/cm (i.e. 50 mM phosphate sodium). When elution requires the addition of organic solvents, linear gradients corresponding to 10 cv should be used. Care must be taken with organic solvents due to the potential protein denaturation or explosive conditions.

Cleaning and maintenance

In order to avoid frequent regeneration of the column, use only samples and buffers that are clear and previously filtered (0.22 µm). Make sure that changes in pH and ionic strength to be used during the chromatography do not cause precipitation of sample components.

The following regeneration procedures are recommended for general and specific cleaning challenges:

Situation	Recommendation
General Cleaning-In-Place (CIP)	Wash with low salt buffers at pH 4.0-8.5.
Tightly bound impurities	To be removed by organic solvents such as alcohols, glycols, detergents, or chaotropics such as guanidine-HCl and urea.
When the impurities to be eliminated are not known	Wash with a sodium hydroxyde solution (0.1 - 0.5 M). 1 to 5 cv of this solution are generally sufficient to remove alkaline soluble material.
After treatment	Neutralize the column immediately by washing with a strongly (> 200 mM) buffered solution (e.g. Tris-HCl or acetate).