SARTORIUS

Instructions for Use

HA Ultrogel®

Hydroxyapatite Chromatography Resin

1 Product Description

HA Ultrogel® resin is a hydroxyapatite agarose composite resin for the separation of biomolecules from research and development scale to manufacturing. HA Ultrogel® is a cross-linked tridimensional composite based on spherical agarose beads with entrapped microcrystals of hydroxyapatite.

Hydroxyapatite chromatography is considered to be a pseudo-affinity chromatography, or mixed-mode ion exchange. It has proven to be an effective purification mechanism in a variety of processes, providing biomolecule selectivity complementary to more traditional ion exchange or hydrophobic interaction techniques. HA Ultrogel® is easy scalable and is currently used in research scale to multi-liter column applications.

HA Ultrogel® is available ready-to-use suspended in 1 M sodium chloride with 20% ethanol as bacteriostatic.

2 Properties

Particle size	60 – 180 µm
Hydroxyapatite content (weight | vol.)	40%
Agarose (weight | vol.)	4%
Exclusion limit	> 5,000,000 dt
Working and cleaning pH	5 – 13
Capacity for cytochrome C (50% BT)[(1)]	≥ 7 mg/mL
Capacity for BSA (50% BT)[(2)]	≤ 7 mg/mL

[(1)] Determined using 5 mg/mL cytochrome C diluted 50/50 in 10 mM sodium phosphate buffer, pH 6.8 at 30 cm/h.
[(2)] Determined using 1 mg/mL BSA diluted 50/50 in 10 mM sodium phosphate buffer, pH 6.8 at 12.5 cm/h.

3 Preparation of the Column

3.1 Choice of the Column

For best results in gradient elution, select a column geometry with a height | diameter ratio between 1 and 6. If the ratio is less than 1, it may be difficult to apply the sample homogeneously. A ratio higher than 6 does not improve the separation of protein mixtures in continuous gradient.

For scale up, it is recommended to increase the diameter of the column to get a higher throughput at optimized linear velocities.

3.2 Preparing Resin for Column Packing

1. Gently agitate the container in which the resin 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 vacuum flask of a 2 cv capacity.
3. Add 0.4 resin volume of deionized water to the resin and gently stir to achieve a homogenous suspension.
4. Gently stir the suspension with a plastic paddle to get a homogeneous slurry and connect the vacuum flask to a vacuum pump to remove dissolved gas.

3.3 Column Packing

HA Ultrogel® is compatible with traditional low pressure chromatography columns and equipment. A column equipped with an adjustable flow adapter facilitates optimal packing and is recommended.

3.3.1 Preparing the Chromatography System

1. Prime the solvent delivery system, lines and valves to assure that all air is displaced. Assure that the top and bottom frits or nets are fully wetted and free of air. Fill the column with packing buffer (sodium phosphate, pH 6.8 is recommended) and operate the system over a range of flow rates representative of values that will be used during packing and anticipated chromatographic procedures. Record pressure associated with the empty column and system (including detectors, etc...).
2. Determine pressure | flow characteristics for the empty system, taking into account recommendations concerning flow rate during packing and chromatographic operation.
3. Stop the pump, close the column outlet, and remove the upper flow-adaptor or piston.
4. Open the outlet and drain buffer from the column, leaving 1 – 4 cm (0.4 – 1.6 in.) of buffer above the bottom frit.
5. Adjust the pump using the recommended flow rate (see Table below).

Flow Rate Guidelines for HA Ultrogel®

Column Dimensions		Flow rate (cm/h)			
cm	inches	Packing	Equilibration	Working	Regeneration
1.6 x 5	0.6 x 2	250	< 225	5 – 20	110 – 225
1.6 x10	0.6 x 4	150	< 135	5 – 20	70 – 135
2.5 x 5	1 x 2	180	< 160	5 – 30	80 – 160
2.5 x 10	1 x 4	110	< 100	5 – 30	50 – 100
5 x 10	2 x 4	80	< 70	10 – 45	40 – 70
5 x 20	2 x 8	55	< 50	10 – 45	25 – 50

3.3.2 Packing the Column

1. Gently stir the previously equilibrated HA Ultrogel® resin slurry to obtain a homogeneous suspension.
2. Pour the slurry into the column in one continuous motion. In order to minimize introduction of air bubbles, pour the slurry along a glass rod held against the column.
3. Gently stir the slurry in the column using a plastic rod or paddle.
4. Allow the suspension to settle for 5 – 10 min until a clear supernatant layer (~1 cm / 0.4 in.) is visible at the top of the column.
5. Insert the upper plunger into the column and carefully adjust to ensure that no air is trapped under the net or frit. Avoid turbulence or other disturbance of the resin. Connect the inlet to the working buffer.
6. Open the column outlet and operate the pump at the chosen flow rate. When the bed height is stabilized and no further compaction is observed, stop the pump and close the column outlet.
7. Re-position the plunger so that 1 – 4 mm (0.04 – 0.2 in.) of buffer is visible between the plunger and the top of the bed.
8. Repeat step 6. If further compression of the bed occurs, reposition the plunger.
9. Make a final adjustment of the plunger so that the plunger is just in contact with the bed, leaving no visible space between the frit and bed at any point around the circumference.

4 Working Conditions

After packing the column, run 3 cv of working buffer of the same composition as that chosen for chromatography through the resin. At the end of equilibration, make sure that the ionic strength and pH are identical at both the outlet and inlet.

4.1 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. Start simultaneously the pump and the recorder.

At the process scale, typical flow rates from 30 to 200 cm/h are currently applied with multi-liter column sizes.

4.2 Choice of the Elution Gradient

Either a continuous gradient or a discontinuous step gardient can be used.

4.2.1 Continuous Gradient

Use a gradient maker (automatic or manual). The first solution is constituted of the diluted working buffer (10 mM sodium phosphate, pH 6.8), the second solution is the concentrated buffer (0.5 M sodium phosphate, pH 6.8). Before making the gradients, perform the chromatography by elution with 2 – 3 cv of the first buffer (isocratic separation).

4.2.2 Discontinuous Step Gradient

Run through the column predetermined volumes of phosphate buffers, each having a higher molarity than that used before. The recommended volumes to be used for each buffer is 3 cv.

5 Cleaning and Maintenance

In order to avoid frequent regeneration, use only samples and buffers previously filtered (0.2 µM). Make sure that changes in pH and ionic strength to be used during the chromatography do not cause precipitation of sample components.

After repeated uses and if necessary, HA Ultrogel® may be regenerated in column or in batch to restore its efficiency and dynamic sorption capacity.

Acidic washings must be avoided in all cases.

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

Situation	Recommendation
General Cleaning-In-Place (CIP)	Wash with 2 cv of 500 mM sodium phosphate buffer, pH 6.8. In most cases, this treatment is sufficient to clean the resin.
Adsorbed material	Wash with: — 1 – 2 M sodium chloride in sodium phosphate buffer 0.5 M, at pH around neutrality (never use acidic buffers at pH < 4), 1 – 2 cv. — 0.1 M sodium hydroxide, 1 – 2 cv, 1 hour contact time — Detergents such as Triton™ X-100* (0.1 – 1%), 1 cv. — Dissociating agents such as urea (4 – 6 M), 1 – 2 cv.

After regeneration, HA Ultrogel® must be washed with 0.5 M sodium phosphate buffer, pH 6.8 and reequilibrated in the starting buffer. After sanitization, the column must be reequilibrated with sterile, pyrogen-free running buffer or storage solution.

⚠ **Warning**
Never wash HA Ultrogel® with acidic solutions which dissolve the crystals of hydroxyapatite. Do not use chelating agents such as EDTA, EGTA, citrate etc., which decrease the adsorption capacity of the resin. However, these agents may be useful to desorb components very strongly bound to the resin, in this case HA Ultrogel® cannot be regenerated.

6 Thermal Stability and Storage

Temperature of use	2 – 30 °C (36 – 86 °F)
Storage temperature	2 – 30 °C (36 – 86 °F) (2 – 8 °C | 36 – 46 °F once opened)
Recommended storage solution (between runs)	Neutral buffer containing bacteriostatic agents such as 1 M NaCl and 20% (v/v) ethanol.
⚠	Product must never be frozen
i	Product is shipped at ambient temperature

7 Ordering Information

Pack Size	Part Number
25 mL	24775-082
100 mL	24775-025
500 mL	24775-017
1 L	24775-041
10 L	24775-058
20 L	24775-066

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Sartorius Stedim Chromatography Resins SAS
48 avenue des Genottes
F-95800 Cergy Saint Christophe
www.sartorius.com

Code: 290188-V01
Status: September 2024