Bio-Gel® HT
Bio-Gel HTP
DNA Grade Bio-Gel HTP
Hydroxyapatite

Instruction Manual

BIO-RAD

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Section 1
Properties of Bio-Gel HT, HTP, and DNA Grade HTP Hydroxyapatite

Hydroxyapatite, a crystalline form of calcium phosphate, is widely used in preparative biochemistry, having proven itself a unique tool for the fractionation and purification of monoclonal antibodies[1-7] and other proteins,[8,9] enzymes,[10-12] and nucleic acids.[13-18] Hydroxyapatite is useful for preparative work in column or batch modes, and for quantitative analysis of proteins or nucleic acids. Its advantages include:

Unique selectivity - Since molecular separation on hydroxyapatite is not primarily dependent on molecular weight, molecular size, charge density, or isoelectric point, hydroxyapatite chromatography is a valuable complement to other separations techniques.*

* For a discussion of the mechanism of action of hydroxyapatite, refer to the publications of M. J. Gorbunoff.[19-21]

High capacity - Hydroxyapatite has a high capacity for nucleic acids and proteins. Its surface area is about 50 m^2 per gram.

Low non-specific adsorption - Non-specific adsorption of hydrophobic substances is minimized by the inorganic crystalline matrix of hydroxyapatite $[Ca_5(PO_4)3OH]_2$. Hydroxyapatite displays negligible adsorptive capacity for low molecular weight substances such as mononucleotides, salts, and amino acids.

Chemical and thermal stability - The wide range of chemical compatibilities (aqueous and inorganic solvents), the thermal stability (autoclavable), and the pH tolerance (pH >5.5) permit the use of hydroxyapatite under conditions that optimize the binding of nucleic acids and proteins.

Economy - The initial cost of the material is low, and can be used several times.

Commercial hydroxyapatite preparations may vary considerably in their ability to achieve the desired chromatographic resolution. All Bio-Rad hydroxyapatite is tested for separation of double-stranded DNA from

single-stranded DNA, albumin binding capacity, DNA binding capacity, and flow capacity. The test results for ds DNA from ss DNA for each batch are printed on the package label.

1.1 Bio-Gel HT Fully Hydrated Hydroxyapatite

Bio-Gel HT hydroxyapatite, prepared by the method of Tiselius, *et al.*,[22] is shipped suspended in 10 mM sodium phosphate buffer containing 0.02% NaN_3. This material gives excellent resolution at a high flow rate (see Table 1) because of its large particle size. Bio-Gel HT hydroxyapatite has a shelf life of at least 1 year when stored at 4 °C in the shipping buffer.

1.2 Bio-Gel HTP Powder

Bio-Gel HTP hydroxyapatite is the Tiselius material which has been dried by a unique process developed at Bio-Rad. It may be stored without refrigeration, and, when resuspended in buffer, it has the same properties as Bio-Gel HT hydroxyapatite.

1.3 DNA Grade Bio-Gel HTP Hydroxyapatite

DNA Grade Bio-Gel HTP hydroxyapatite, supplied in a dry powder form, has a smaller particle size which significantly increases its capacity and enhances its selectivity for double-stranded DNA molecules. RNA capacity should also be increased, making DNA Grade Bio-Gel HTP hydroxyapatite useful for DNA-RNA hybridization studies. Due to its slower flow rates, it is recommended for batch chromatography or very short columns.

Table 1. Hydroxyapatite Product Performance

	Bio-Gel HT Hydroxyapatite	Bio-Gel HTP Hydroxyapatite	DNA Grade Bio-Gel HTP Hydroxyapatite
Flow rate cm/h column cross section[a]	25-100	35-100	>5
Mg BSA sorbed per dry gram[b]	10	10	10
µg calf thymus DNA sorbed per dry gram	500	500	800
Storage	4 °C	dry form @ room temp. hydrated @ 4 °C	dry form @ room temp. hydrated @ 4 °C

a. Flow rate determined in a 1.5 x 10 cm column with 40 cm H20 hydrostatic pressure.

b. Batchwise uptake.

c. The DNA capacities listed are lower than previously reported due to a change in testing methodology. This reporting change does not represent diminished Hydroxyapatite product preformance or changes to the manufacturing process. The new testing methodology results in a more accurate determination of DNA capacity.

Section 2
Rehydrating Bio-Gel HTP and DNA Grade Bio-Gel HTP Hydroxyapatite

1. Determine the amount of dry powder needed to fill the column. When hydrated, Bio-Gel HTP hydroxyapatite occupies approximately 2-3 ml per dry gram.
2. Add one part Bio-Gel HTP hydroxyapatite or DNA Grade HTP hydroxyapatite to six parts of starting buffer with gentle swirling. Do not use magnetic stir bars or stirring rods, as these will damage the hydroxyapatite crystals.

Note: All buffer should be degassed prior to the addition of the dry hydroxyapatite.

3. Allow the slurry to settle for at least 10 minutes. Then decant the fines which are in the cloudy upper level and at the top of the settled bed. Decant to the settled bed.

4. A second decantation is usually not necessary. If it is desired, add an equal volume of starting buffer to the bed and mix by swirling gently.
5. Repeat step 3 and resuspend a final time for column pouring.

Section 3
Resuspending Bio-Gel HT Hydroxyapatite

1. Bio-Gel HT hydroxyapatite is shipped in 10 mM sodium phosphate buffer, pH 6.8, containing 0.02% NaN_3. It tends to pack in the bottle during shipping, and requires resuspension for column pouring. If a buffer other than phosphate is to be used in the actual elution, it should also be used in the decanting steps. Pour off the phosphate buffer in the bottle and add the new buffer solution before suspending the gel. Buffer pH should remain greater than 6.0.

2. Swirl the buffer in the bottle gently until the gel is in suspension. Do not use stirring rods or magnetic stir bars.
3. After the gel is suspended, pour it into a beaker and allow the gel to settle for at least 30 minutes. Decant the fines which are in the cloudy upper level and at the top of the settled bed. Decant to the settled bed.

Section 4
Pouring the Column

Many types of columns and bed supports are available. Glass barrel Econo-Column® chromatography columns* are useful for hydroxyapatite chromatography.

To pack the column, attach a wide mouth funnel to the top of the column and add the starting buffer to it. Then pour the suspended hydroxyapatite into the funnel and allow 2-3 cm to settle under gravity. Then open the column outlet, and allow the gel to pack under flow.

* For information on Econo-Column low pressure chromatography columns, see Bio-Rad's current catalog.

When the bed is stable, pass at least two bed volumes of starting buffer through the column.

Section 5
Applying the Sample

A load between 1 and 5 mg of protein per ml bed volume is normally used, although much larger amounts have sometimes proved satisfactory. Phosphate buffers are used with a stepwise or gradient increase in concentration while the pH is held constant. Generally speaking, the higher the phosphate concentration the less strongly the proteins are adsorbed.

Initial experiments are conveniently performed by adsorbing the protein in 10 mM buffer and eluting the sample with stepwise concentration increases of about two-fold. If the effluent is not monitored, at least one and one-half column volumes of buffer should be used for each step to provide complete elution of each peak. The void volume of hydroxyapatite is about 75% of the bed volume.

Section 6
Regenerating the Column

A 0.4 M phosphate buffer is usually sufficient to remove adsorbed materials. If a contaminated sample has been run, it is advisable to remove the top layer of the hydroxyapatite bed and wash the remainder of the bed with one bed volume of 1 M sodium chloride followed by four column volumes of starting buffer.

For information on Bio-Rad's high performance hydroxyapatite (Bio-Gel HPHT) columns, request bulletin 1115, or contact your local Bio-Rad representative. In the U. S., call technical service at 1-800-4BIORAD.

Section 7
References

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Section 8
Ordering Information

Catalog Number	Product Description	Pkg. Size
130-0150	**Bio-Gel HT (Hydrated) Hydroxyapatite**	250 ml
130-0151	**Bio-Gel HT (Hydrated) Hydroxyapatite**	500 ml
130-0420	**Bio-Gel HTP (Powder) Hydroxyapatite**	100 g
130-0520	**DNA Grade Bio-Gel HTP (Powder) Hydroxyapatite**	100 g
737-6201	**Thermal Chromatography Column,** for DNA hydroxyapatite chromatography, 1 x 30 cm jacketed Econo-Column chromatography column and 2 flow adaptors	

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