BIOSEPRA® HA ULTROGEL®

HA Ultrogel®

Hydroxyapatite Chromatography Sorbent

- Effective purification mechanism in a variety of processes
- High porosity
- Easy cleaning
- Used in large scale step

Biosepra HA Ultrogel sorbent available from Ciphergen is a hydroxyapatite agarose composite sorbent for the separation of biomolecules from research and development scale to manufacturing.

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 scaleable and is currently used in research scale to multi-liter column applications.

HA Ultrogel hydroxyapatite sorbent is composed of cross-linked agarose beads with microcrystals of hydroxyapatite entrapped in the agarose mesh. The particle size ranges between 60 and 180 µm. The agarose moiety in HA Ultrogel is chemically stabilized with epichlorohydrin under strongly alkaline sorbent. Thus HA Ultrogel can be regularly treated with 0.1-1.0 M NaOH for regeneration and sanitization. This creates glycerol bridges between the polysaccharide chains and gives the sorbent beads an excellent rigidity and stability to pH and ionic strength changes, as well as to high temperature.

HA Ultrogel porosity is comparable to an agarose gel, with an exclusion limit for globular proteins of 5,000,000 daltons. This macroporosity avoids any moleculear sieving effect during the separation (see figure 1).

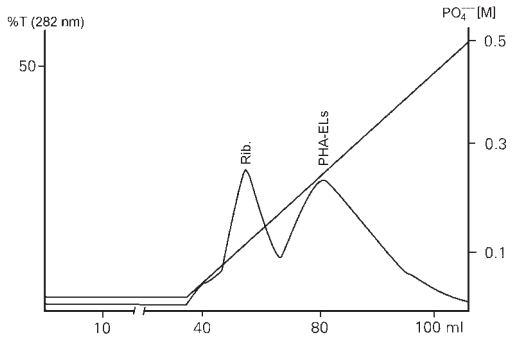
The sorbent is shipped in 1 M NaCl containing 20% ethanol and is available in a range of package sizes. Special packaging to meet specific manufacturing requirements is available on request.

Table 1: HA Ultrogel Main Properties.

Particle size	60-180 µm
Hydroxyapatite content	40 %
Agarose (weight/volume)	4 %
Exclusion limit	> 5,000,000 dt
Working pH	5-13
Cleaning pH	5-14
Thermal stability	4-121°C
Capacity for cytochrome c*	> 7 mg/ml
Capacity for BSA**	< 7 mg/ml

* Determined using 5 mg/ml cytochrome c diluted 50/50 in 1 mM phosphate buffer, pH 6.8 at 30 cm/h.
** Determined using 1 mg/ml BSA diluted 50/50 in 1 mM phosphate buffer, pH 6.8 at 12.5 cm/h.

Figure 1. Separation of a mixture of ribonuclease and PHA-ELs.



Column: 1.6 x 6.5 cm; Sample: 1 mg of protein mixture composed of ribonuclease (MW 14,700) and PHA-ELs (Erythroagglutinating and lymphostimulating Phytohemagglutinin) (MW 128,000) from Phaseolus vulgaris, in 1 ml of 5 mM potassium phophate, pH 6.8; Elution gradient: 5 mM to 500 mM potassium phosphate, pH 6.8; Flow rate: 14.4 cm/h.

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Stability

The recommended flow rates to be used with HA Ultrogel sorbent depend on the column geometry and on the separation phase (capture, elution or washing steps). At process scale, typical flow rates from 30 to 200 cm/h are currently applied with multi-liter column sizes.

Hydroxyapatite crystals are naturally resistant to most chemical agents, except solutions with a pH less than 4 and complexing agents. Hydroxyapatite is dissolved by acidic solutions, while EDTA, citrate and other complexing agents decrease the adsorption capacity of the resin. Complexing agents may be used in extreme cases, e.g. when the desorption of certain compounds irreversibly bound to the matrix is required.

HA Ultrogel sorbent is resistant to denaturing agents: it can be treated with 8 M urea, 6 M guanidine-HCl, 1% SDS and chaotropic agents such as 3 M KSCN.

The agarose moiety of HA Ultrogel sorbent is chemically stabilized by cross-linking with epichlorohydrin in a strong alkaline medium. HA Ultrogel sorbent is stable in alkaline conditions, and can be regularly treated with 0.1 to 1M sodium hydroxide for regeneration and depyrogenation. The chromatographic behavior of the sorbent was not significantly modified of after 5 weeks of incubation in 1M NaOH, pH 13.

HA Ultrogel sorbent should not be treated with solutions at pH <4 due to the nature of the hydroxyapatite crystals.

HA Ultrogel sorbent is stable a high temperature (up to 121°C). It can be sterilized by autoclaving without undergoing any changes to its chromatographic properties. However, the operation should be performed in buffered conditions at pH 7 to avoid the presence of phosphate which may precipitate.

HA Ultrogel sorbent should never be frozen.

Applications

Hydroxyapatite adsorption chromatography can be used in a variety of applications, including the separation of proteins, peptides and nucleic acids,from pilot to production scale (see figures 2, 3, 4).

For proteins, the most well-known application of hydroxyapatite is the separation of basic proteins (cytochrome c, lysozyme, etc.) and phosphoproteins. HA Ultrogel sorbent can be used for the separation of human serum proteins and plant proteins such as lectins, glycoproteins, glycosidases, phospholipidases, sulfohydrolases, sphingomyelinases, transferases, trehalases and kinases.

As a phosphate-containing sorbent, HA Ultrogel can be used for the separation of phosphate-dependent proteins and enzymes as well as DNA-dependent enzymes.

HA Ultrogel sorbent provides an efficient tool for IgG purification in a one step chromatographic purification by separation with phosphate buffer. This approach is very mild (neutral pH, physiological conditions) compared to traditional elution in acidic solutions, and preserves the biological activity of the antibody.

HA Ultrogel has been used for the separation of:

- Synthetic polypeptides (acidic polypeptides such as poly-L-glutamate, poly-L-aspartate).
- Basic polypeptides such as poly-L-lysine, poly-L-ornithine.
- Neutral polypeptides such as poly-L-proline.

It can be used for the separation of various types of nucleic acids, including transfer RNA and low molecular weight glyoxylated derivatives of DNA, with reproducibility, stability and reliability (see figure 2 and 3).

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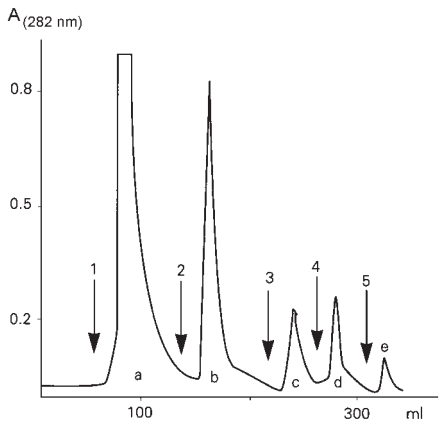
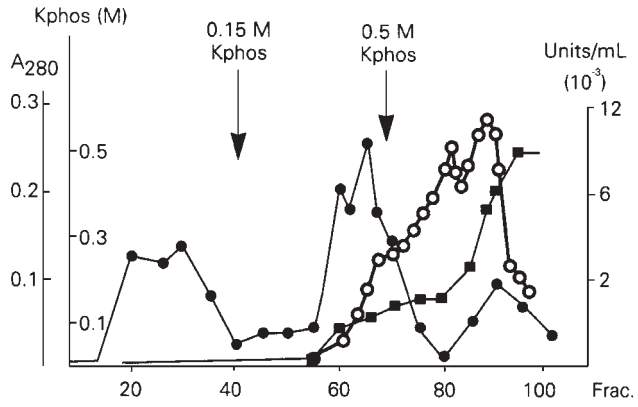
Figure 2. Separation of glycohydrolases from a crude enzyme extract of buckwheat.



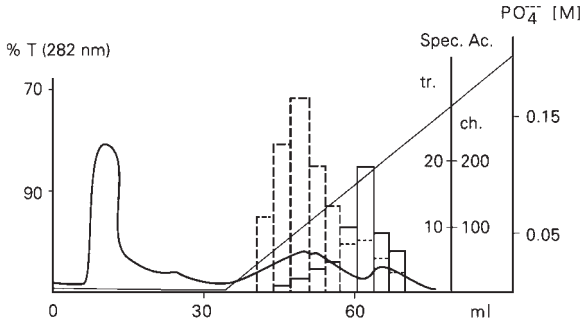
Column: 2 x 6 cm; Sample: 40 mg of lyopholized extract in 1 ml of 1 mM phosphate buffer, pH 6.8; Discontinuous elution gradient of phosphate buffer; Flow rate: 7.1 cm/h; Temperature: 4°C. Peak a: proteins with no glycohydrolase activity; peak b: β-glucosidase. Courtesy of R. Rourbouze & F. Percheron, Biochemistry Lab., Faculty of Pharmacy, Paris.

Figure 3. Purification of damage-specific DNA binding protein from human placenta.



The sample was previously prepurified from human placenta by ammonium sulfate precipitation, ion exchange chromatography and gel filtration.
Column: 2.5 x 6 cm; Initial buffer: 10 mM potassium phosphate, pH 8 containing 5% glycerol and 13 mM 2-mercapto-ethanol; 1st elution (arrow): 0.15 M potassium phophate buffer; 2nd (arrow): 0.5 M potassium phosphate buffer; Fraction volume: 1.8 ml.
●—● $A_{280\ nm}$ ■—■ DNA binding activity o—o conductivity.
Courtesy of R.S. Feldberg, et al., J. Biol. Chem. 257 (1982) 6394-401.

Figure 4. Separation of trypsin and chymotrypsin from a porcine pancreatic enzyme extract.



Column: 1.6 x 5 cm; Sample: 30 mg protein in 1 ml of 5 mM phosphate buffer, pH 6.8; Gradient: 5 to 200 mM sodium phosphate, pH 6.8; Flow rate: 10 cm/h; Temperature: 10°C;
Histogram with broken line: trypsin activity; Histogram with solid line: chymotrypsin activity.
Spec. Ac.: specific activity in U/mg. tr: trypsin, ch: chymostrypsin. Trypsin activity was primarily found in the peak eluted by 50 mM phosphate where the chymotrypsin was eluted by 100 mM phosphate. The final yield was approximately 50%.

References

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Ordering Information

Product	*Cat. No.*	*Size*
HA Ultrogel	24775-025	100 ml
	24775-017	500 ml
	24775-041	1 L
	24775-058	10 L
	24775-066	20 L

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About BioSepra® Products & Collaborative Services

Ciphergen Biosystems develops, manufactures and markets BioSepra® process chromatography sorbents that greatly simplify protein purification development and significantly improve biopharmaceutical manufacturing productivity.

Over the past 25 years, BioSepra® chromatography products and services have earned an outstanding reputation for product innovation and technical support. Our expanded R&D sorbent program, new ISO 9001 manufacturing plant and recently launched MEP HyperCel® represent our latest commitment to the biopharmaceutical industry.

With the acquisition of BioSepra® products and services, Ciphergen has been able to combine chromatography development expertise with SELDI-based ProteinChip® technology to set in motion an entirely new approach to protein purification called Process Proteomics. With the addition of ProteinChip® technology, sample analysis and purification development are combined. This single-step, on-chip approach dramatically accelerates and simplifies purification development and analysis. The future of Process Proteomics begins with Ciphergen.

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