LT BIOTECH UAB,
Mokslininku g. 6A,
LT-08412 Vilnius,
Lithuania
www.ltbiotech.lt

Pleiad CHT (Ceramic Hydroxyapatite)

1. Basic product information

Hydroxyapatite [$Ca_{10}(PO_4)_6(OH)_2$] is an inorganic pure phase chromatography resin, which is calcined into spheres at high temperature. Its crystal structure is the same as that of bone hydroxyapatite; the spherical structure is stable and the particle size is uniform. There are multiple modes of interaction between hydroxyapatite and biomolecules, which can achieve two separation mechanisms of cation exchange and calcium metal affinity simultaneously. The cation exchange mainly depends on the negatively charged phosphate groups on hydroxyapatite. At the same time, carboxyl clusters and phosphorus clusters on biomolecules can form with calcium on hydroxyapatite through a metal-affinity, stronger bond. Due to these unique separation mechanisms, hydroxyapatite is an indispensable chromatographic resin in today's extremely demanding downstream processes in antibodies, vaccines (such as bacterial polysaccharides, pneumococcal polysaccharides and viruses), nucleic acids, enzymes and recombinant proteins, playing an important role in the field of biopharmaceuticals.

2. Product parameters

Item	Type I	Type II
Type	Multimodal chromatography resin: cation exchange, calcium affinity	
Functional group	Ca^{2+}, PO_4^{3-}, -OH	
Resin particle size	Available in 20, 40, 60, 80 μm	
Dynamic binding capacity*	25–60 mg mAb/ml	15–25 mg mAb/ml
Maximum work pressure	30 bar	40 bar
Recommended flow rate	50–400 cm/h	

** Measurement conditions: flow rate 300 cm/h; test buffer 10 mM PBS, pH 6.8.*

3. Chemical resistance

pH stability*	6.5–14
Chemical stability	All commonly used aqueous buffers, 1M NaOH, 6M guanidine hydrochloride, 8M urea, ethanol, methanol, 100% acetonitrile. Note: The pH of guanidine hydrochloride aqueous solution with different concentrations can be as low as 4.6, and the aqueous solution of urea can also be acidic due to impurities; pH ≥ 6.5 should be ensured during use. Avoid using chelating agents like EDTA, EGTA and citrate ions.

LT BIOTECH UAB,
Mokslininku g. 6A,
LT-08412 Vilnius,
Lithuania
www.ltbiotech.lt

4. Method of use

4.1 Chromatographic conditions
(1) Buffer selection: buffer salts whose buffer groups do not interact with the chromatography resin should be selected. If the binding and elution mode is used, the equilibration buffer should be a low pH (usually 1 pH unit lower than the isoelectric point of the target molecule, but the pH should not be lower than 6.5) to facilitate the binding of the target molecule. Consider the stability of the sample in the buffer; the elution buffer is usually a buffer with a high concentration of salt (such as 1-2M NaCl) added to the equilibration buffer.
(2) Flow rate: generally, choose a linear flow rate of 90–500 cm/h according to the bed height of the column.
(3) Sample pretreatment: to prevent the sample from clogging the column, the sample needs to be filtered with a 0.45 μm microporous membrane before loading.

4.2 Chromatography steps
(1) Pre-equilibration: use pre-equilibration buffer (for example: 0.4 M Na_3PO_4, pH 6.8) to quickly and fully equilibrate the column to the corresponding pH. This step usually requires 3–4 CV.
(2) Equilibration: use equilibration buffer (for example: 0.005 M Na_3PO_4, 0.1M NaCl, pH 6.8) to fully equilibrate the column until the pH and conductivity are stable and basically consistent with the equilibration buffer; this step usually requires 5-10 CV.
(3) Loading*: determine the loading volume and loading amount of the sample on the Pleiad CHT according to the binding capacity measured in the small test experiment.
(4) Washing*: use equilibration buffer or other suitable buffer to wash the column until the UV stabilises and returns to the baseline.
(4) Elution*: elution is achieved by increasing the concentration of salt ions or phosphate ions, which can be gradually increased by linear gradient or step gradient (for example: linear gradient 0–100%, 0.005M Na_3PO_4, 1-2M NaCl or 0.1M NaPB, pH 6.8), to elute molecules with different binding strengths. Fraction collection was performed on the eluted samples.
(4) Regeneration: flush the column with a high-concentration phosphate solution (such as 0.4M Na_3PO_4 pH 8.0). This step typically requires 5 CV.
(5) Cleaning: rinse the column with 0.005 M NaPB buffer pH 7.0. Reduce the concentration of phosphates to prevent the formation of bottoms.
* If the flow-through mode is used, the ‘sample loading’ step should be set to collect; the ‘washing’ step should ensure that all the target molecules have flowed through, then the collection can be stopped; the ‘elution’ step should directly use high-salt buffer – impurities can be washed away.

5. Cleaning and sterilisation

Contaminants (such as lipids, endotoxins and proteins) accumulate on the column as the number of uses of the chromatography resin increases. Regular cleaning-in-place (CIP) is essential to keep the column in a stable working condition. Determine the frequency of CIP based on the degree of contamination of the chromatography resin (if contamination is considerable, it is recommended that CIP is performed after each use to ensure reproducible results and to prolong the working life of the chromatography resin).
For different types of impurities and contaminants, the recommended cleaning conditions are as follows:
• Removal of stronger binding proteins: wash with 5 CV of 2M NaCl solution.
• Removal of strongly hydrophobic proteins and precipitated proteins: first wash with 5 CV of 1M NaOH solution, and then use 5–10 CV of 0.1M NaOH to decrease lye concentration.
• Removal of lipoproteins and lipids: first wash with 5 CV of 70% ethanol or 30% isopropanol, then rinse with 5–10 CV of 0.005 M NaPB buffer pH 7.0.

LT BIOTECH UAB,
Mokslininku g. 6A,
LT-08412 Vilnius,
Lithuania
www.ltbiotech.lt

Note: 70% ethanol or 30% isopropanol should be degassed before use; the flow rate can be selected as 30-60 cm/h during CIP; reverse cleaning can be used when the blockage is severe.

To reduce the microbial load, it is recommended that 1M NaOH solution is used to treat the chromatography resin; treatment time is 15–30 minutes. Dry or wet sterilisation can also be used, as long as the solution is still alkaline at high temperature.

6. Storage

Keep the unopened chromatography resin in the original container and store at 4~30°C in a well-ventilated, dry and clean place. Do not freeze. Wash the used column with 2–3 CV of 1M NaOH solution and store at 2~8°C.

7. Destruction and recycling

Since chromatography resin is difficult to degrade in nature, it is homologous to bone and has no effect on the natural environment. For chromatography resin that have been in contact with biologically active samples such as viruses and blood, please follow the local biosafety requirements before destroying or disposing of them.

8. Ordering information

Pleiad CHT type I, 20µm

Product Cat. No	Package
800-00010	10 g
800-00025	25 g
800-00100	100 g
800-01000	1 kg
800-05000	5 kg

Pleiad CHT type I, 40µm

Product Cat. No	Package
802-00010	10 g
802-00025	25 g
802-00100	100 g
802-01000	1 kg
802-05000	5 kg

Pleiad CHT type I, 60µm

Product Cat. No	Package
803-00010	10 g
803-00025	25 g
803-00100	100 g
803-01000	1 kg
803-05000	5 kg

LT BIOTECH UAB,
Mokslininku g. 6A,
LT-08412 Vilnius,
Lithuania
www.ltbiotech.lt

Pleiad CHT type I, 80 µm

Product Cat.No	Package
804-00010	10 g
804-00025	25 g
804-00100	100 g
804-01000	1 kg
804-05000	5 kg

Pleiad CHT type II, 20 µm

Product Cat.No	Package
805-00010	10 g
805-00025	25 g
805-00100	100 g
805-01000	1 kg
805-05000	5 kg

Pleiad CHT type II, 40 µm

Product Cat.No	Package
806-00010	10 g
806-00025	25 g
806-00100	100 g
806-01000	1 kg
806-05000	5 kg

Pleiad CHT type II, 60 µm

Product Cat.No	Package
807-00010	10 g
807-00025	25 g
807-00100	100 g
807-01000	1 kg
807-05000	5 kg

Pleiad CHT type II, 80 µm

Product Cat.No	Package
808-00010	10 g
808-00025	25 g
808-00100	100 g
808-01000	1 kg
808-05000	5 kg