TI-CHA 20/40/80 A/B Hydroxyapatite chromatography Resin

Product Manual

楚天微球生物技术(长沙)有限公司
TRUKING MICRO-SPHERE BIOTECHNOLOGY (CHANGSHA) CO., LTD

Content

1. Product Introduction

Hydroxyapatite for TI-CHA chromatography is a form of calcium phosphate, formed by high temperature calcination of porous spherical material, is a pure inorganic chromatography medium specially designed for the purification of biomolecules, through a variety of mechanisms with biomolecules,

1) the phosphate root is negatively charged, and can be combined with positively charged biomolecules by the formation of ionic bonds;

2) the calcium ions can be combined by the formation of metal chelating bondsbinding to biomolecules with free carboxyl groups, imidazole rings, and other electron donors;

3) hydroxyl groups on hydroxyapatite can bind biomolecules by forming hydrogen bonds with hydroxyl groups on biomolecules.

Spherical hydroxyapatite calcined at lower temperatures has smaller pores and a relatively large specific surface area, known as type A hydroxyapatite, which has a higher binding capacity for smaller molecular weight biomolecules and is mainly used to purify most proteins (molecular weights below 100 kd). Type B hydroxyapatite calcined at a higher temperature forms larger internal pores, which have a higher binding load for larger biomolecules such as antibodies and nucleic acids, making it suitable for the purification of antibodies. Meanwhile, type B has a greater retention of nucleic acids, and is able to discriminate between single- and double-stranded, super-helical, and other kinds of structural DNA, and thus is also suitable for the purification of nucleic acids.

Since the crystal structures formed at different temperatures vary, the binding specificity for biomolecules is significantly different.

Table 1: TI-CHA technical parameters

	TI CHA A type			TI CHA B type		
Model	CHA-20A	CHA-40A	CHA-80A	CHA-20B	CHA-40B	CHA-80B
Particle Size	20μm	40μm	80μm	20μm	40μm	80μm
Appearance	White powder (dry)					
Chemical composition	$Ca_{10}(PO_4)_6(OH)_2$					
Standard pore size	600-800Å			800-1000Å		
Dynamic Binding Load	>25mg lysozyme/g medium Approx. 30mg human IgG/g media			>12.5 mg lysozyme/g media Approx. 20mg human IgG/g media		
Chemical Stability	1M NaOH, 8M urea, 6M guanidine hydrochloride, ethanol, acetonitrile					
Maximum flow rate	300cm/h（20μm），600cm/h（40μm），1200cm/h（80μm），TK-EC16，h=10cm					
Maximum pressure resistance	10MPa					
pH stability	6.5-12 (work) 6.5-14 (CIP, short-term)					
Density	0.63g/ml					

2. Methods of use

2.1 Chromatography column loading

- Calculate the amount of TI-CHA hydroxyapatite needed based on the volume of the chromatography column, i.e., the volume needed multiplied by the density of 0.63 g/ml is the weight needed.
- Add 3 times the volume of distilled water for washing, hot water can be used to facilitate the removal of air bubbles that are not within the medium.
- Gel suspension is prepared by adding one-half to one times the volume of distilled water of the settling gel and stirring well.
- Take the cleaned chromatography column, drain the membrane air bubbles at the bottom of the column, and keep a water column about 1cm high at the bottom of the column, adjust the column so that it is perpendicular to the ground.
- Pour the stirred colloidal suspension into the chromatographic column slowly at one time, pay attention to not bring in air bubbles, stir again with a plastic rod after pouring, and let it stand for 30min to let the medium settle naturally and completely.
- Connect the upper column head to the chromatographic system or peristaltic pump, drain the air bubbles under the screen of the upper column head (for the chromatographic column with diameter less than 20cm, you can use the peristaltic pump or the washing ball to suck out the air bubbles under the screen after turning the column head upward), put the column head into the chromatographic column, shake the column head so that the air bubbles can be discharged from the edge of the column head, and tighten the sealing knob (for the chromatographic column with diameter >30cm, the sealing ring should be put into the column first, and then the sealing ring will be put into the column first.Put the column head into the chromatography column, shake the column head so that air bubbles can be discharged from the edge of the column head, then tighten the sealing knob (for the chromatography column with diameter >30cm, do not tighten the sealing ring too much, press the column head down to let the liquid inside the column head back out to discharge air bubbles from the column head, then tighten the sealing knob).
- Give a certain flow rate to press the rubber surface, because CHA is very pressure-resistant and has no stretching, so the column bed does not drop significantly under the flow rate.
- Stop the pump, close the bottom outlet, loosen the head seal slightly, make sure the valve on the head is open, press down on the head to the glue surface, tighten the head seal, and the installation of the column is complete.

2.2 Column Effectiveness Evaluation

Column efficiency can be determined by using acetone as indicator or NaCl as indicator, and the indicator solution and mobile phase are prepared according to the following table.

Table 2: Column efficiency determination methods

Methods	Acetone Method for Column Efficacy	Column Efficacy by NaCl Method
Sample	1.0% (v/v) acetone in water	0.8M NaCl (dissolved in water)
Sample volume	1.0% column volume	1.0% column volume
Mobile phase	Water	0.4M NaCl aqueous solution
Flow rate	30 cm/h	30 cm/h
Detection Data	UV 280 nm	Conductivity

2.3 Calculating Column Effect

Theoretical plate height (HETP), theoretical number of plates (N) and asymmetry factor (As) were calculated from the UV or conductivity curves with the following equations:

HETP=L/N

$N=5.54(V_R/W_h)^2$

Where: V_R = retained volume

W_h=half peak width

L=column height

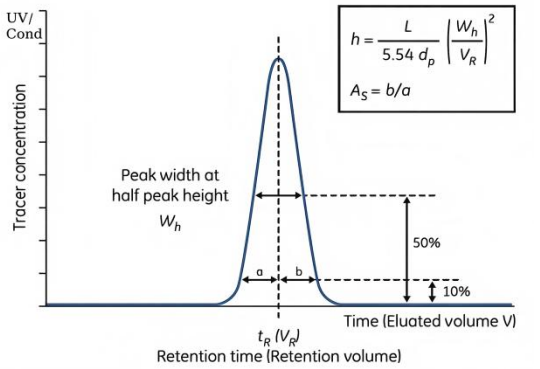
N=theoretical plate number

The units of V_R and W_h should be the same;

As=b/a

Where: a= first half peak width at 10% peak height

b= second half peak width at 10% peak height



2.4 Evaluation of results

h=HETP/d_{50v}

d_{50v} = median particle size volume distribution (cm)

The h-value calculated by the above formula is less than 3, and the asymmetry factor is 0.8~1.8 then it is judged to be qualified. For unsatisfactory column efficiency the reason needs to be analyzed and the column reloaded.

2.5 Chromatographic methods

Since there are various mechanisms and modes of CHA binding to biomolecules, it needs to be tested by several condition explorations to get better results, here is just an introduction of the initial recommended chromatographic conditions:

- Regardless of acidic and basic proteins, type A of TI-CHA has high loading. type B of TI-CHA binds basic proteins better and has low selectivity for acidic proteins.
- Buffer: A common binding buffer is 5 mM phosphate, pH 6.8. Basic proteins can be eluted linearly or in phase with 1 M NaCl phosphate buffer, pH 7.2.Acidic proteins can be eluted linearly or in phase with 500 mM phosphate buffer.It is critical that both binding and elution buffers be above pH 6.5, avoiding buffers containing EDTA and citrate.
- Sample and sample volume: To prevent the sample from clogging the column, the sample needs to be filtered through a 0.45μm microporous filter membrane and the pH and conductivity of the sample adjusted to be consistent with the equilibration buffer before sampling.
- Flush the chromatography column with equilibration buffer until the UV absorption curve is smooth.
- Elution:

1, sodium chloride gradient elution: using 10mM PB containing 1MNaCl buffer as eluent, linear elution or step gradient elution, this elution can elute the biomolecules that are bound to CHA by ion exchange.

2, phosphate gradient elution, using 0.2M phosphate buffer, linear elution or stepwise gradient elution, this elution method can elute the biomolecules bound to CHA through metal interaction.

- Note: Very few proteins do not bind at 5 mM phosphate or are not eluted by 500 mM phosphate.If this occurs, lower the pH or reduce the phosphate concentration to 1 mM, but keep the pH above pH 6.5 and add calcium ions to the buffer.Note, however, that using less than 5 mM phosphate will result in reduced column life.

3. Cleaning and regeneration

Regeneration: Specific regeneration programs are designed based on sample and media contamination.

- If the sample is relatively clean and the chromatography column is not heavily contaminated you can use a buffer rinse with 1M NaCl in 0.2M phosphate.
- 0.5M NaOH can be used to remove some impurities.
- 6M guanidine hydrochloride or 8M urea can be used to remove more heavily contaminated chromatography columns, especially if there is protein precipitation.
- Rinse off the cleaning reagent with 5 column volumes of purified water.
- Equilibrate the chromatography column with equilibration buffer and set aside.

4. Sterilization and storage

TI CHA hydroxyapatite can be autoclaved at 121 degrees or treated with 1M NaOH for 0.5-1h to achieve sterilization.

TI CHA Hydroxyapatite is stored in 0.1M sodium hydroxide, and to prevent a drop in alkali concentration, it is recommended that the preservation solution be replaced with a fresh one once every 3 months.

5. Destruction and recycling

Since TI CHA hydroxyapatite is a non-toxic, non-hazardous, natural inorganic compound, it can be disposed of as general solid waste after eliminating biological contamination.

6. Ordering Information

Table 3 Article number and packaging

Product	Item No.	Norm	Product	Item No.	Norm
TI CHA-20A	Y5337	25g	TI CHA-20B	Y5343	25g
	Y5338	100g		Y5344	100g
	Y5339	500g		Y5345	500g
	Y5340	1kg		Y5346	1kg
	Y5341	5kg		Y5347	5kg
	Y5342	25kg		Y5348	25kg
TI CHA-40A	Y5349	25g	TI CHA-40B	Y5355	25g
	Y5350	100g		Y5356	100g
	Y5351	500g		Y5357	500g
	Y5352	1kg		Y5358	1kg
	Y5353	5kg		Y5359	5kg
	Y5354	25kg		Y5360	25kg
TI CHA-80A	Y5361	25g	TI CHA-80B	Y5367	25g
	Y5362	100g		Y5368	100g
	Y5363	500g		Y5369	500g
	Y5364	1kg		Y5370	1kg
	Y5365	5kg		Y5371	5kg
	Y5366	25kg		Y5372	25kg