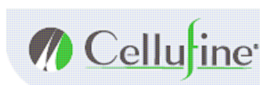


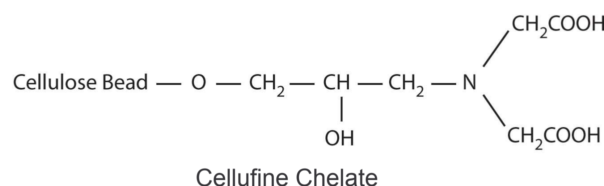
Affinity Chromatography

Cellufine Chelate

Cellufine is the liquid chromatography media for the purification of proteins, enzymes and other bio-active substance. Since it is made from spherical cellulose particles having high chemical stability, high mechanical strength and bio-compatibility, it is suitable for the production in pharmaceutical and food industry. And the leaking from this matrix is much less than that from the synthetic polymer media.

Introduction

Cellufine Chelate is designed for immobilized metal chelate affinity chromatography of proteins and peptides. The packing comprised of spherical cellulose beads to which iminodiacetic acid (IDA) has been immobilized. Its superior rigidity allows high flow rates and thus, rapid processing times. When exposed to metal salts, IDA readily complexes with the cation. The resulting metal chelate moiety interacts (primarily) with the surface accessible histidine residues.



Cellufine Chelate

Features & Benefits

- High flow rates in laboratory and process columns, allowing high throughput
- Low ligand leakage
- Low non-specific adsorption
- Autoclavable and chemically stable : Easy cleaning and depyrogenation (0.5 M NaOH)
- Exclusion limit : >4000 kDa : Compatible with large molecular weight proteins
- Spherical cellulose beads : Easy packing
- Group specific affinity applicable for various proteins

Physical-Chemical Characteristics

Support matrix	Cellulose
Particle shape	spherical
Particle diameter (µm)	125 – 210
Zn^{2+} adsorption capacity (µM/ml)	22 - 30
Cu^{2+} adsorption capacity (µM/ml)	35 - 45
MW exclusion limit (kD)	4,000
PH stability range	2 - 12
Operating pressure	< 2 bar (29 psi)
Supplied	suspension in 20 % EtOH

Instructions for Use

Column Packing

1. Calculate volume required for the desired bed dimension.
2. Prepare a 40 – 60 % (v/v) slurry with the appropriate adsorption buffer. Allow the packing to equilibrate at ambient temperature for one hour.
3. Gently stir or place under vacuum to degas.
4. With column outlet closed, carefully pour the slurry into column. Depending on the volume, a filler tube may be necessary.
5. With the inlet open to release air, insert and affix the top adjuster assembly at the slurry interface.
6. Open the column outlet and begin pumping adsorption buffer at rate 10 % - 20 % higher than the operational flow rate.
7. After the bed stabilizes, close the column outlet. Then, with the inlet open, reposition the end cell on top of the bed. Equilibrate with 10 column volumes of adsorption buffer before sample loading.

Operating Guidelines

General Operation

1. Load with the appropriate metal ion (Ca^{2+}, Zn^{2+}, Ni^{2+} or Co^{2+}).
2. Equilibrate with adsorption buffer.
3. Load the sample.
4. Wash the column to remove unbound contaminants.
5. Elute bound proteins.

Metal ion Loading

Prepare a 50 mM solution of the appropriate metal salt ($CaCl_2$, $ZnCl_2$, $NiCl_2$, $CoCl_2$ etc.) in DIW ; ensure that the pH is 6 – 8. Flush the column with 10 bed columns of the metal salt solution. Then, wash with the same quantity of adsorption buffer.

Recommended Buffers

Adsorption buffer : in general adsorption is performed at pH 7.5 in 10mM phosphate buffer containing 0.2 – 1.0 M NaCl. High ionic strength promotes adsorption.
Elution buffer : bound sample can be eluted by one of these methods :

- lowering pH (e.g., pH 4 – 5)
- displacement by imidazole, ammonium chloride, etc. (100 mM)
- the addition of chelating agents (50mM EDTA, citric acid, etc.)

The pH method should be attempted, first followed by various combinations of the others. Generally, step gradient elution is most effective for preparative applications.

Sample Preparation and Load

Prepare the sample in the appropriate adsorption buffer. Remove any insoluble material by centrifugation or microfiltration. Load the sample onto the column and wash with 5 – 10 bed volumes of adsorption buffer. The sample should not contain chelating substances such as EDTA or citric acid.

Flow Rate

The recommended linear velocity range for Cellufine Chelate is 20 – 150 cm/h.

Chemical and Physical Stability

Compatible with most salts, detergents and chaotropic agents. The operating pH range is 3 – 12. Autoclavable in suspension at neutral pH for 30 minutes at 121°C.

Regeneration and Depyrogenation

Cellufine Chelate is typically regenerated with either 2 – 4 column volumes of 50 mM EDTA in 0.5 M NaCl, pH 7.0. The packing can be depyrogenated with a few bed volume washes of 0.2 N NaOH. In either case, the column must be re-loaded with metal cation before further use.

Storage

Short term (2 weeks or less), bulk and column can be stored at room temperature with 0.05 N NaOH. Longer storage should be in neutral buffer containing 0.02 % sodium azide or 20 % ethanol at 4 – 8°C. Do not freeze.
Shelf Lifetime : 5 years

References

Janson, J.-C. and Ryden, L., *Protein Purification: Principles, High Resolution Methods, and Applications.* 2nd ed. New York: John Wiley & Sons, Inc., 1998

Produit	Quantité	Référence
Cellufine Chelate	**10 ml**	**676951324**
Cellufine Chelate	**50 ml**	**19875**
Cellufine Chelate	**500 ml**	**19876**

for research use only, not intended for diagnostic use.