Affinity

Cellufine Amino

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.

Description

Cellufine Amino is a primary amine activated support for the covalent immobilization of carboxyl containing proteins and ligands. As with all Cellufine products, the base support consists of spherical cellulose beads which exhibit superior rigidity and chemical stability relative to classical agarose gels. Such mechanical strength allows for improved throughput at both the bench scale and on the production floor. The exclusion properties of Cellufine Amino are similar to those of 4 % agarose gels. Furthermore, due to the robust internal structure and chemical linkage, immobilized ligands show no appreciable leakage. With the use of a condensation agent, ligands (protein, etc.) can be easily coupled via the reactive amine moiety.

Physical-Chemical Characteristics

Support matrix	cellulose
Particle shape	spherical
Particle diameter (µm)	125 – 210
Active group	Primary amine (- NH_2)
Amino density (µM/ml)	15 - 20
Protein capacity (mg/ml)	Up to 40
MW exclusion limit (kD)	4,000
Density (ml/g-damp medium)	1.3
Operating pressure	< 1 bar (14.5 psi)
Supplied	suspension in 20 % EtOH

Coupling

The coupling of the ligands having carboxyl groups to Cellufine Amino proceeds via the reaction below:

//- CH_2- CHOH-CH_2-NH_2 + HOOC - (Protein) + R'- N = C = N - R'

→ //- CH_2- CHOH-CH_2-NH-CO- (Protein) + R' - NH - CO - NH – R'

Materials

- Coupling buffer : 0.1 M NaCl, adjusted to pH to 4.5 ± 0.5 with HCl.
- Carbodiimide condensation agents : the most common is 1-ethyl-3- (dimethyl aminopropyl) - Carbodiimide hydrochloride (EDAC). EDAC is water soluble and most suitable for ordinary coupling reactions. Another reagent which can be used is dicyclohexylcarbodiimide (DCC). However, this reagent generates a water insoluble byproduct which requires an organic solvent (e.g. EtOH) for removal.
- Ligand solution : typically, 1 – 20 mg/ml in coupling buffer. After the ligand solubilizes, check that the pH is 4.5 ± 0.5. If multiple concentrations are being evaluated, vary by 5 mg/ml intervals.
  Note : total mass coupled will be directly related to concentration.

General Procedure

Perform the following in a suitable mixing vessel.

1. Estimate the required volume of medium.
2. Wash the medium with 5 volumes of coupling buffer. This is accomplished by suspending the medium in the buffer and allowing to sit for several minutes, then decant and discard the buffer supernant. Repeat 2 times.
3. Add the ligand solution at a 1:1 volume ratio (settled medium: ligand solution). This will form a total slurry volume approximately double the original volume of medium.
4. Based on total slurry volume, add solid EDAC to a concentration of 10 mg EDAC/ml slurry. Stir or swirl solution of EDAC and medium slurry to ensure solubilization.
5. Gently stir for 5 hours at room temperature.
6. Wash 3 times with 5 volumes of chromatographic adsorption buffer (e.g., 20 mM sodium phosphate/0.1 M NaCl).

Column Packing

1. Calculate volume required for the desired bed dimension, keeping in mind that some compression of the bed will occur during column packing.
2. Prepare a 40 – 60 % (v/v) slurry with the appropriate adsorption buffer.
3. With outlet closed, pour the slurry into column. Depending on the volume, a filler tube may be necessary.
4. With the inlet open to release air, insert and affix the top adjuster assembly at slurry interface.
5. Open the column outlet and begin pumping adsorption buffer at rate at least 20 % higher than the operational flow rate.
6. After the bed stabilizes, close the column outlet. Then with the inlet open, reposition the end cell on top of the bed.

Operating Guidelines

General Operation

1. Wash column with 5 bed volumes of elution buffer.
2. Equilibrate with 5 bed volumes of adsorption buffer.
3. Load sample.
4. Wash with 5 bed volumes of adsorption buffer.
5. Elute sample with 5 bed volumes of elution buffer

Recommended Buffers

Conditions will depend on the ligand used. However, the following mobile phases are generally useful for immuno-affinity chromatography.
Elution buffer : 0.1 M glycine, pH 3.5

Sample Preparation and Load

Prepare a 1 – 10 mg/ml solution of sample in adsorption buffer. Remove insoluble material by centrifugation or microfiltration.

Flow Rate

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

Chemical and Physical Stability

The stability of the coupled gel will be limited by the ligand. However, the base medium is stable to most salts, detergents, chaotropic agents, 0.1 N NaOH, 0.1 N HCl and can be autoclaved at 121□ for 30 minutes, (pH 7.0).

Regeneration

The regeneration protocol will depend on ligand stability. In some cases, a few bed volume washes with elution buffer containing 0.1 % Tween®20 or Triton® X-100 will be sufficient. A similar wash with 6 M urea can also be effective.

Storage

Cellufine Amino should be stored at 4°C in 20 mM phosphate, 20 % EtOH, 0.1 M NaCl, pH 7.2 containing 0.02 % sodium azide (assuming that the coupled ligands is stable under such conditions).

Produit	Quantité	Référence
Cellufine Amino	10 ml	676945324
Cellufine Amino	50 ml	19856

for research use only, not intended for diagnostic use.