

## Operating Instructions

### Affinity Chromatography Media Cellufine Amino

#### Description

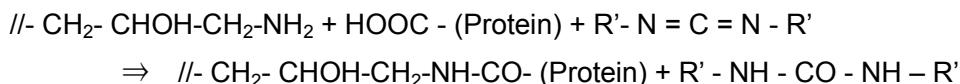
Cellufine Amino is a primary amine activated support for the covalent immobilization of carboxyl containing proteins and ligands. As with all Cellufine products, the base support consists of spherical cellulose beads which exhibit superior rigidity and chemical stability relative to classical agarose 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 ( $\mu$ m)	125 – 210
Active group	Primary amine (- NH <sub>2</sub> )
Amino density ( $\mu$ M/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:



### Materials

- Coupling buffer: 0.1 M NaCl, adjusted to pH to  $4.5 \pm 0.5$  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  $4.5 \pm 0.5$ . 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 supernatant. 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°C 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).

**Product Ordering Information** (Catalogue No.)

Media type	Quantity (liters)				
	0.01	0.05	0.5	5	10
Cellufine Amino	6769 45324	19856	19857	19858	6769 45335

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## Affinity Chromatography Media

### Cellufine Formyl

#### Description

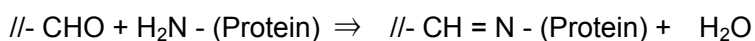
Cellufine Formyl is an aldehyde activated support for the covalent immobilization of amine containing proteins and ligands. As with all Cellufine products, the base support consists of spherical cellulose beads which exhibit superior rigidity and chemical stability relative to classical agarose gels. Such mechanical strength allows for improved throughput at both the bench scale and on the production floor. The exclusion properties of Cellufine Formyl 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 (proteins, etc.) can be easily coupled via the reactive aldehyde moiety.

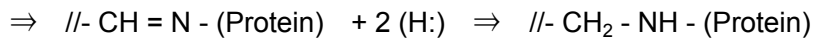
#### Physical-Chemical Characteristics

Support matrix	cellulose
Particle shape	spherical
Particle diameter ( $\mu$ m)	125 – 210
Active group	aldehyde
Aldehyde density ( $\mu$ M/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 protein ligands having primary amino groups to Cellufine Formyl proceeds via a Schiff's-base intermediate followed by reduction with either NaCNBH<sub>3</sub> (SCBH) or NaBH<sub>4</sub> (SBH) as illustrated below:





### Materials

- Coupling buffer: 20 mM sodium phosphate, 0.1 M NaCl, pH 7.0.
- Blocking buffer: 4 M Tris, pH 7.0
- Reducing agent: the most common reducing agents are sodium cyanoborohydride (SCBH) and sodium Borohydride (SBH). Prepare a 75 mg/ml solution either.

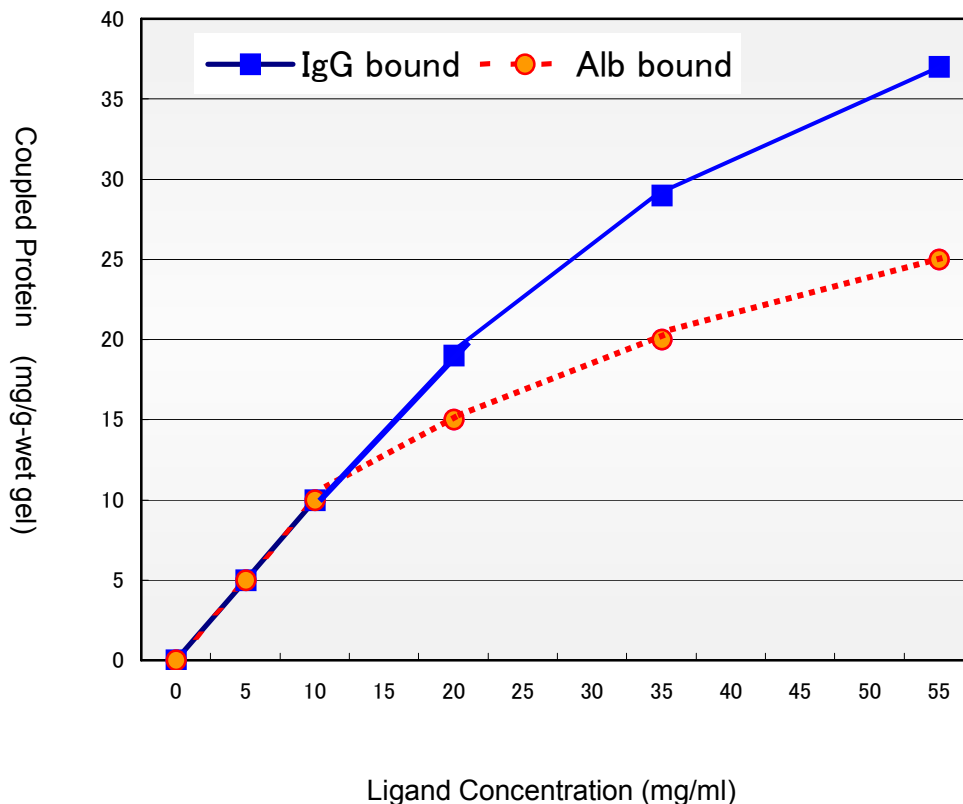
**Caution:** Solution of SCBH should be made in a well ventilated area, since a small amount of toxic gas will be generated upon solubilization.

**Note:** SCBH is reasonably specific for Schiff 's bases.

- Ligand solution: typically, 1 – 20 mg/ml in coupling buffer. If multiple concentrations are being evaluated, vary by 5 mg/ml intervals.

Note: total mass coupled will be directly related to concentration (see Fig. 1).

**Fig. 1 Effect of Ligand Concentration**



### General Coupling Procedure

Perform the following in a suitable mixing vessel.

7. Estimate the required volume of medium.

8. Wash the medium with 5 volumes of coupling buffer. This is accomplished by suspending the medium in the buffer and allowing it to sit for several minutes, then decant and discard the buffer supernatant. Repeat 2 times.
9. 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.
10. If using SCBH, add reducing agent at a quantity  $1/20^{\text{th}}$  that of the total volume. Mix gently for 4 hours at room temperature.
11. If using SCBH as the reducing agent, add it after the medium and ligand mixture has “incubated” for 4 hours and allow it to sit for an additional hour (after adding the SBH) at room temperature.
12. At the completion of the reduction (in either case), add a quantity of blocking buffer (4 M Tris, pH 8) equivalent to  $1/20^{\text{th}}$  of the total solution volume.
13. Gently stir for 1 hour at room temperature.
14. Wash 3 times with 5 volumes of chromatographic adsorption buffer (e.g., 20 mM sodium phosphate/0.1 M NaCl).

### **Coupling Considerations**

Ligand loading and biochemical activity are influenced by solute concentration, pH, reaction time and temperature. The coupling conditions above will be appropriate for most applications.

### **Ligand Concentration**

Ligand loading is directly related to the ligand concentration. Using a 50 % slurry (1 ml gel per 1 ml ligand solution) and about 10 mg/ml ligand concentration, 60 % coupling can be achieved in approximately 4 hours, at room temperature. Higher efficiency can be obtained with longer reaction times.

### **Schiff's base Formation and Reduction**

A Schiff's base is readily formed between the amine ligand and aldehyde-gel. Sodium cyanoborohydride specifically reduces this linkage to a C - N bond, minimally affecting the ligand. Given such specificity, sodium cyanoborohydride can be added at time zero. In contrast, if  $\text{BaBH}_4$  is used there should be a pre-incubation period in order to allow Schiff's base formation. Otherwise, the aldehyde moiety will be prematurely reduced.

#### **Reaction Temperature**

Lower temperature result in lower reaction rate and in some cases, less efficient coupling. As such, recommended reaction time should be increase to 16 hours, if refrigeration at  $4^{\circ}\text{C}$  is required.

## **Column Packing**

7. Calculate volume required for the desired bed dimension, keeping in mind that bed compression will occur during column packing.
8. Prepare a 40 – 60 % (v/v) slurry with the appropriate adsorption buffer.
9. With outlet closed, pour the slurry into column. Depending on the volume, a filler tube may be necessary.
10. With the inlet open to release air, insert and affix the top adjuster assembly at slurry interface.
11. Open the column outlet and begin pumping adsorption buffer at rate at least 20 % higher than the operational flow rate.
12. 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**

6. Wash column with 5 bed volumes of elution buffer.
7. Equilibrate with 5 bed volumes of adsorption buffer.
8. Load sample.
9. Wash with 5 bed volumes of adsorption buffer.
10. Elute sample with 5 bed volumes of elution buffer

### **Recommended Buffers**

These conditions will depend on the ligand used. However, the following are generally useful for immuno-affinity chromatography.

Adsorption buffer: 20 mM phosphate, 0.1 M NaCl (pH 7.2). Depending on application, other buffer ions may be used.

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 Formyl is 20 – 150 cm/h.

### Chemical and Physical Stability

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

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