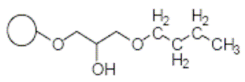

## Hydrophobic Interaction

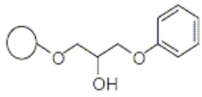
# Cellufine Butyl, Phenyl

Cellufine

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.




Cellufine Butyl




Cellufine Phenyl

**Description**

Cellufine Butyl & Phenyl are useful for the chromatography of hydrophobic proteins. Many proteins have hydrophobic amino acid residues which will interact with the phenyl, butyl and octyl functional groups. Factors that influence this hydrophobic interaction include salt concentration, temperature, pH, organic solvents and surfactants. Protein adsorption usually occurs at high ionic strength, while elution occurs at lower salt concentrations.
This is the opposite of ion exchange chromatography and complementary.

**Physical-Chemical Characteristics**

| | Butyl | Phenyl |
|---|---|---|
| Support matrix | cellulose | |
| Particle shape | spherical | |
| Particle diameter (µm) | 53 – 125 | |
| BSA capacity (mg/ml) | > 11 | > 12 |
| BSA elution efficiency (%) | > 50 | > 50 |
| MW exclusion limit (kD) | 4,000 | |
| pH stability range | 2 - 13 | |
| pH operating range | 1 - 13 | |
| Operating pressure | < 1 bar (14.5 psi) | |
| Supplied | suspension in 20 % EtOH | |

**Column Packing**

1. Calculate volume required for the desired bed dimension.
2. Prepare a 40 – 60 % (v/v) slurry in 50 mM sodium phosphate, 1 M $(NH_4)_2SO_4$, at pH 7.0.
3. Gently stir or place under vacuum to degas.
4. With column outlet closed, carefully pour the slurry into the 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 column outlet and begin pumping buffer at a 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 adsorption buffer before sampling.

## Operating Guidelines

**General Operation**

Equilibrate column with 2 – 5 volumes of elution buffer (low salt concentration), then wash with the same amount of loading buffer. The typical loading buffer is 50 mM of sodium phosphate, pH 7.0 containing 0.5 – 2.5 M $Na_2SO_4$, $(NH_4)_2SO_4$ or NaCl. Adsorption strength is a function of salt concentration, pH and temperature. In general, high concentration promotes adsorption. Desorption (elution) is then accomplished by lowering the salt concentration. Fore more information, see References.

**Sample Preparation and Load**

Samples are ideally prepared in loading buffer. Filtration may be required to remove insoluble matter. If necessary, buffer exchange may be accomplished using diafiltration or desalting chromatography. Protein adsorption and recovery will vary with each packing. Usually, in terms of binding strength, Cellufine Phenyl > Butyl. The sample (prepared in the loading buffer) is applied after the loading buffer wash. Once the sample is loaded, flush with 5 column volumes of loading buffer to remove unbound material. Subsequently, bound product can be eluted.

**Flow Rate and Elution**

The recommended linear velocity range for hydrophobic Cellufine media is 50 – 200 cm/h.
Generally, flow rates at the lower end of this range are ideal for binding and elution, while higher flow rates are acceptable for washing and regeneration steps.
Desorption of bound material is accomplished by step elution with low concentration of salt (e.g., less than 0.5 M). The use of chaotropic agents (e.g., KSCN), surfactants (e.g., Octyl Glucoside, CHAPS, Triton® X, Chaps or Tween®) (e.g., ethanol) will improve the recovery of tightly adsorbed proteins.

**Chemical and Physical Stability**

PH 2 – 13, when operated at room temperature. Stable in most salts (NaCl, $(NH_4)_2SO_4$, etc.) and most detergents (SDS, Tween, Chaps, etc.) Can be cleaned using 0.2 N NaOH.
**Autoclavable** in suspension at neutral pH for 20 minutes at 121°C.

**Regeneration**

Flush the column with 2 - 5 bed volumes of 0.2 N NaOH. In some cases, an additional flush with 2 - 5 bed volumes of 70 % EtOH/30 % DIW /0.1 M AcOH followed by distilled water may be required to remove adsorbed lipids.

**Storage**

Short term (2 weeks or less), bulk and column can be stored at room temperature with 2 M $(NH_4)_2SO_4$ or 0.05 N NaOH. Long term storage should be in neutral buffer containing 0.02 % sodium azide or 20 % EtOH in refrigerator at 4 – 8°C. Do not freeze.

**Shelf Lifetime** : 5 years

**References**


*1. Harris, E.L.V. and Angal, S., Protein Purification Methods: A practical Approach. New York: Oxford University Press, 1989.*

*2. 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 Butyl** | **100 ml** | **19905** |
| **Cellufine Butyl** | **500 ml** | **19906** |
| **Cellufine Phenyl** | **100 ml** | **19900** |
| **Cellufine Phenyl** | **500 ml** | **19901** |

**for research use only**, not intended for diagnostic use.


interchim 211 bis Av. J.F. - BP 1140 03103 Montluçon Cedex - Tel. 33 (0) 4 70 03 88 55 - Fax 33 (0) 4 70 03 82 60
e-mail interchim@interchim.com - web www.interchim.com