USER GUIDE: Hydrophobic Interaction Chromatography

This material is for the following products:

EBL HIC_Phenyl-50S, HIC_Butyl-50S, and HIC_Octyl-50S

Introduction:

- Hydrophobic interaction chromatography (HIC) is a versatile method for the purification and separation of biomolecules based on their surface hydrophobicity. HIC can be used as a first purification step or as the final polishing step to remove remaining impurities. EBL HIC resin Pheny, Butyl, and Octyl media substances are separated on the basis of their varying strength of theirhydrophobic interaction with hydrophobic groups attached to the uncharged matrix. EBL HIC media has many properties ideal for a matrix in preparation of chromatographic matrix that is better than agarose-based beads. EBL HIC medium offers the following three features:high performance
- high dynamic binding capacity
- high flow rate

All these features are important for chromatography. All the products manufactured in EBL are based on polyacrylate polymer matrix and with high quality and high batch-to-batch reproducibility, ideal for any stage of an operation – from process development all the way to scale-up and production.

Specifications:

Particle Size (µm)	50 ± 5%
Pore Size (Å)	800
Bead Matrix	Highly cross-linked polyacrylate polymer
Ligand Density	≈50 µmol/mL resin
Suggested Flow Rate (cm/h)	150-700
pH Stability	3-13
Pressure (MPa)	10
Chemical Stability	1.0M NaOH, 30% isopropanol, 70% ethanol, 6M guanidine-hydrochloride, 30% Acetonitrile, 1mM HCl
Storage buffer	20% ethanol
Storage temperature	+4-28°C

Storage:

Use 5 column volumes of distilled water followed by 5 column volumes of 20% ethanol to

wash column. Degas the ethanol/water mixture thoroughly and apply at a low flow rate to avoid over-pressuring the column. Ensure that the column is sealed well to avoid drying out. Store columns and unused media at +4°C to +28°C in 20% ethanol. Do not freeze.

Recommended Procedure and Working Conditions:

Preparing the gel

1. Equilibrate all material to room temperature.
2. Decant the shipping solution and replace it with binding buffer. Note: EBL hydrophobic interaction chromatography come in suspension in 20% ethanol as standard shipping solution.
3. Degas the slurry under vacuum.

Equilibration

To equilibrate, pump approximately 5-8 column volumes of start buffer through the column at a flow rate of 3 to 5 ml/min. The column is fully equilibrated when the pH and/or conductivity of the effluent is the same as the start buffer.

Sample Preparation

The amount of sample that can be applied to the column depends on the available capacity of the hydrophobic interaction chromatography and the degree of resolution required.
The sample should be dissolved in start buffer. Alternatively, the sample may be transferred to start buffer by dialysis or by buffer exchange using a Desalting or a Gel filtration column.
The viscosity of the sample should not exceed that of the buffer. For normal aqueous buffer systems, this corresponds to a protein concentration of approximately 50 mg/ml. Before application the sample should be centrifuged or filtered through a 0.45 µm filter to remove any particulate matter.

Operating flow rates

The flow rate used for sample binding and subsequent elution will depend on the degree of resolution required, but is normally within the range 10 to 15 ml/min. The lower the flow rate, the better the resolution.

Binding

The binding of proteins to hydrophobic media is influenced by:

- the structure of the ligand (e.g., carbon chain or an aromatic ligand)
- the ligand density
- the ionic strength of the buffer
- the salting-out effect
- the temperature

Those salts which cause salting-out (e.g., ammonium sulphate) also promote binding to hydrophobic ligands. The column is equilibrated and the sample is applied in a solution of high ionic strength. A typical starting buffer is 1.7M $(NH_4)_2SO_4$, which is just below the concentration employed for salting out proteins. Hydrophobic interactions are weaker at lower temperatures. This must be taken into account if chromatography is done in a cold room.

Elution

Bound proteins are eluted by reducing the strength of the hydrophobic interaction. This can be done by:

- reducing the concentration of salting-out ions in the buffer with a decreasing salt gradient (linear or step)
- increasing the concentration of chaotropic ions in the buffer with an increasing gradient (linear or step)
- eluting with a polarity-reducing organic solvent (e.g., ethylene glycol) added to the buffer
- eluting with detergent added to the buffer

The Hofmeister series Increasing salting-out effect:
Anions: $SO_4^{2-} > HPO_4^{2-} > CH_3COO^- > Cl^- > NO_3^- > Br^- > ClO_3^- > I^- > SCN^-$
Cations: $NH_4^+ > K^+ > Na^+ > Li^+ > Mg^{2+} > Ca^{2+}$ > guanidinium
Increasing the salting-out effect strengthens hydrophobic interactions; increasing the chaotropic effect weakens hydrophobic interactions. A suggested starting liner gradient is start at 1.7M $(NH_4)_2SO_4$* with 50mM phosphate buffer (pH 7.0) to 50mM phosphate buffer (pH 7.0).
* When working with proteins which have a tendency to aggregate, start with a lower $(NH_4)_2SO_4$ concentration to avoid protein precipitation.

Regeneration

After every run, elute reversibly bound material with low ionic strength buffer at a flow rate of 3 ml/min. Monitor the UV absorbance during regeneration to determine when bound substances have been completely washed out of the column. Wash the column with 5 column volumes of distilled H_2O and re-equilibrate with 5 column volumes of starting buffer. In some applications, substances such as denatured proteins or lipids do not elute in the regeneration procedure. These can be removed by cleaning-in-place (CIP) procedures.

Cleaning-in-place (CIP)

Remove precipitated proteins by washing the column with 5 column volumes of 1M NaOH solution at a flow rate of 1-1.5 ml/min, followed immediately with 5 column volumes of distilled water and re-equilibrate with 5 column volumes of starting buffer.

Remove strongly hydrophobically bound proteins, lipoproteins and lipids by washing the column with 5 column volumes of 70% ethanol or 30% isopropanol. Apply increasing concentration gradients to avoid air bubble formation, when using high concentrations of organic solvents. Wash the column with distilled water and re-equilibrate.

Sanitization

Sanitization can reduce microbial contamination of the medium bed to a minimum. Wash the column in the reversed flow direction for 30-60 minutes with 0.5-1M NaOH, at a flow rate of 1-1.5 ml/min. Re-equilibrate the column with approximately 5 column volumes of sterile start buffer.

Ordering information:

CAT NO:	Product	Quantity
hic-5001100	HIC_Butyl-50S	100 ml
hic-5001500	HIC_Butyl-50S	500 ml
hic-5001504	HIC_Butyl-50S	2000 ml
hic-5002100	HIC_Octyl-50S	100 ml
hic-5002500	HIC_Octyl-50S	500 ml
hic-5002504	HIC_Octyl-50S	2000 ml
hic-5003100	HIC_Phenyl-50S	100 ml
hic-5003500	HIC_Phenyl-50S	500 ml
hic-5003504	HIC_Phenyl-50S	2000 ml