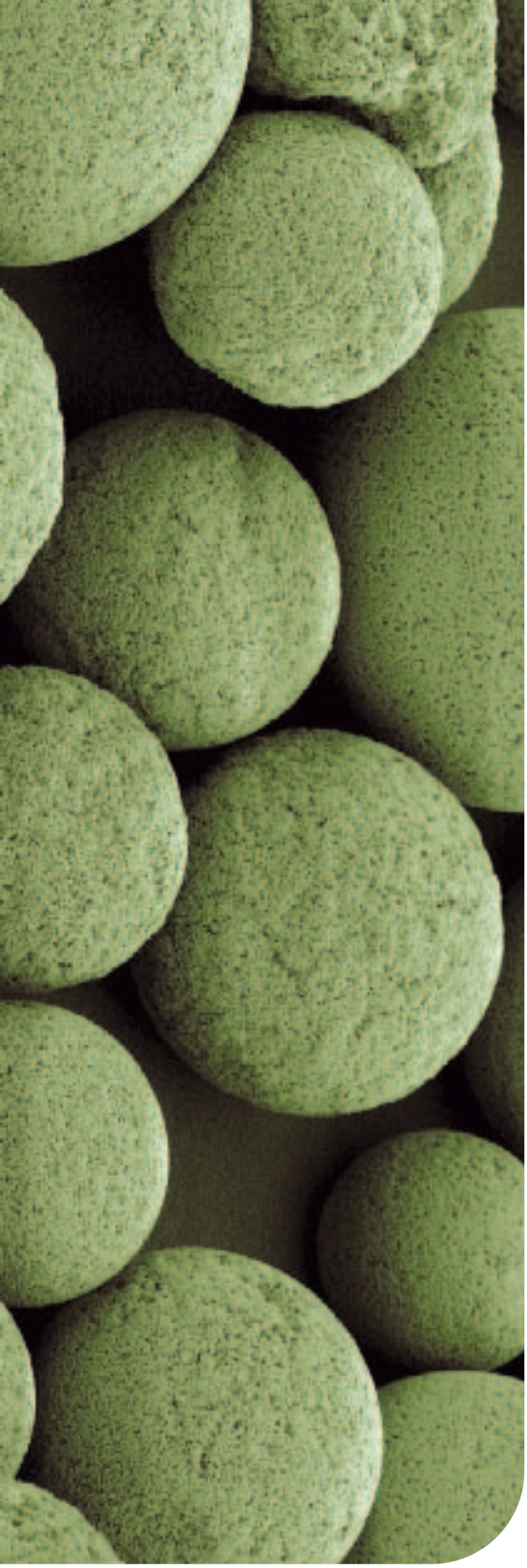


# Fractogel®EMD

Tentacle phases for hydrophobic interaction and thiophilic adsorption chromatography

Advancing Your Life Sciences – From Discovery to Launch

MERCK

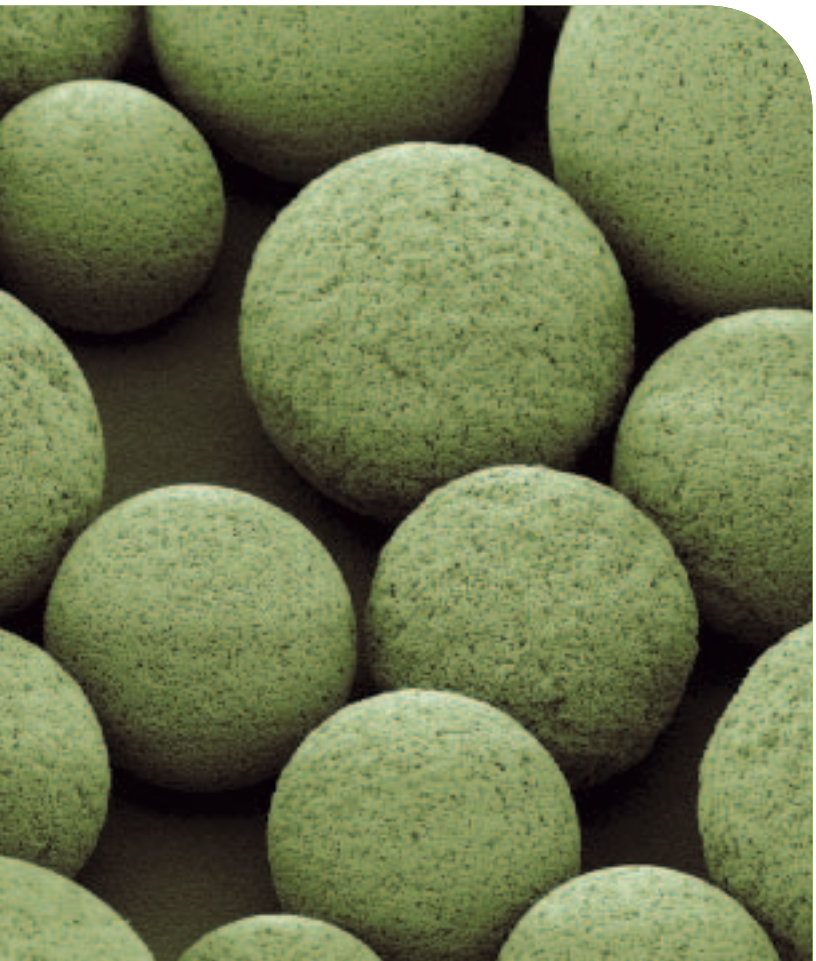
# Fractogel® EMD Phenyl, Propyl and TA

Improve your Return on Investment by:

- ► non-proteinanceous ligands
- ► excellent selectivity and high recovery
- ► outstanding mechanical stability

## Hydrophobic interaction chromatography (HIC) on tentacle media

High performance hydrophobic interaction chromatography is a powerful technique for the isolation of proteins at production scale. The method of hydrophobic interaction chromatography (HIC) is based on the interaction between hydrophobic ligands, fixed on the chromatographic support, and hydrophobic areas located on the surface of proteins. The hydrophobic feature of a given protein is caused by certain amino acid residues like isoleucine, valine, leucine, phenylalanine, and others. Although most of the hydrophobic amino acid residues are located in the centre of the molecule, structure analyses have shown hydrophobic patches on the protein's surface. These areas are often correlated to the biological function of the protein. The hydrophobic character of a protein is promoted by high salt concentrations since a salting out effect can be achieved.



## Advantages of HIC tentacle materials

For the purification of proteins with exposed prominent hydrophobic areas, a chromatography support of moderate hydrophobic character should be used. Both structure and density of the ligands have to be considered with respect to the hydrophobic character of the resin. A moderately hydrophobic material should be used preferably to avoid problems during elution of the protein. Fractogel® EMD Propyl and Fractogel® EMD Phenyl are tentacle media displaying weak and strong hydrophobic features. The high selectivity of Fractogel® EMD HIC-media allows for efficient purification steps **(Fig.1)**. Both phases with a particle size of 20-40 µm are mechanically stable and resistant to biological and chemical degradations. The functional groups are located on linear polymer chains. Fractogel® EMD Phenyl (S) and Fractogel® EMD Propyl (S) are suitable for a wide range of applications, the latter is the material of choice for purification of proteins with a large amount of hydrophobic areas. Due to the weak hydrophobicity of Fractogel® EMD Propyl, a high mass recovery can be expected.

## Your advantages

| | | |
|---|---|---|
| high mechanical strength | ► | easy to pack |
| high pressure stability | ► | up to 80 % ethylene glycol can be used |
| high selectivity | ► | high purity |
| moderate hydrophobicity | ► | high recoveries |

## Ordering information

| Designation | Particle size | Type of chromatography | Content | Ord. No. |
|---|---|---|---|---|
| Fractogel® EMD Propyl (S) | 20 - 40 µm | weak HIC | 100 ml | 1.10085.0100 |
| Fractogel® EMD Propyl (S) | 20 - 40 µm | weak HIC | 500 ml | 1.10085.0500 |
| Fractogel® EMD Phenyl (S) | 20 - 40 µm | strong HIC | 100 ml | 1.16197.0100 |
| Fractogel® EMD Phenyl (S) | 20 - 40 µm | strong HIC | 500 ml | 1.16197.0500 |
| Fractogel® EMD TA (S) | 20 - 40 µm | thiophilic adsorption | 100 ml | 1.16473.0100 |
| Fractogel® EMD TA (S) | 20 - 40 µm | thiophilic adsorption | 500 ml | 1.16473.0500 |

*Larger quantities on request.*
*Additional Merck products for your biopharmaceutical applications:*
*Amino acids; High-quality mineral salts and buffers; Biological buffer substances; ACS grade reagents*
*For more information please visit* www.merck4pharma.com

## Application areas

The main application areas for hydrophobic interaction chromatography are purification of cytosolic proteins, antibodies and recombinant proteins **(Fig. 2)**. Membrane proteins can also be isolated with this technique. For the elution of strong hydrophobic proteins, detergents or organic solvents may have to be used. In addition, HIC is suited for the removal or exchange of non-ionic detergents. Because the presence of high salt concentrations in the buffer does not interfere, hydrophobic interaction chromatography is ideally used in combination with an ion exchange column.

## Adequate Buffers

Due to the weak hydrophobic properties of soluble proteins, the separation on hydrophobic media is performed in the presence of concentrated salt solutions. Hydrophobic interactions are increased by multivalent ions, whereas they are reduced by chaotropic salts. These salts form large monovalent ions that reduce the polarity of the buffer. All buffer systems normally used for hydrophobic interaction chromatography can be used with the tentacle gels. Buffers should be prepared with an appropriate salt concentration. For example, phosphate buffer (20 - 50 mM) can be used containing 0.9 - 1.8 M ammonium sulphate (avoid precipitation of the sample!). Also other salts can be recommended such as various phosphate salts, sodium acetate, or sodium sulfate.

## Elution Conditions

Depending on the selectivity wanted, linear flow rates up to 300 cm/hr can be used. Both tentacle media for hydrophobic interaction chromatography maintain high resolution at high sample loading. Injection of a tenfold amount of protein onto the column does not significantly reduce resolution between ribonuclease A and ovalbumin. Elution can be facilitated by a combined gradient of decreasing salt concentration and increasing concentration (0-80%) of a suitable organic solvent (e.g. ethylene glycol).

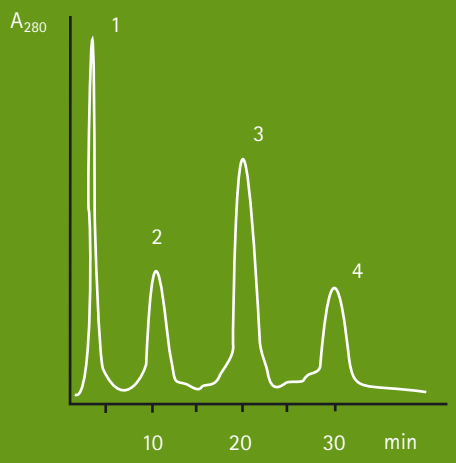



**Fig. 1**
*Separation of cytochrome c (peak 1), myoglobin (peak 2), lysozyme (peak 3) and chymotrypsinogen A (peak 4) on Fractogel® EMD Phenyl (150 x 10 mm column, buffer A: 20 sodium phosphate, 1.8 M ammonium sulfate, pH 7; buffer B: 20 sodium phosphate, pH 7; flow rate: 1ml/min).*

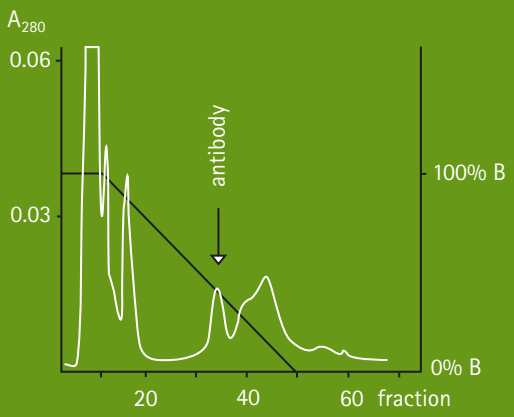



**Fig. 2**
*Separation of a monoclonal antibody on Fractogel® EMD Propyl (S). The antibody was eluted by a gradient of decreasing salt concentration (buffer A: 20 mM sodium phosphatecontaining 0.9 M ammonium sulfate, pH 7.0; buffer B: 20 mM sodium phosphate, pH 7.0; flow rate: 1ml/min).*

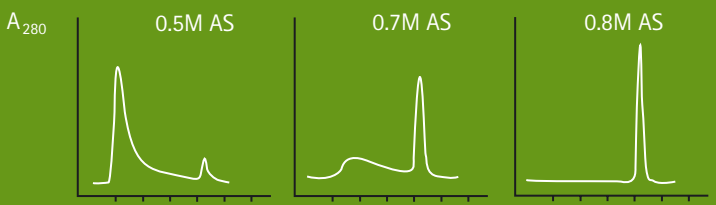



**Fig. 3**
*Salt promoted adsorption of human IgG on Fractogel® EMD TA. Complete binding of immunoglobulin depends on the concentration of ammonium sulphate in the buffer.*

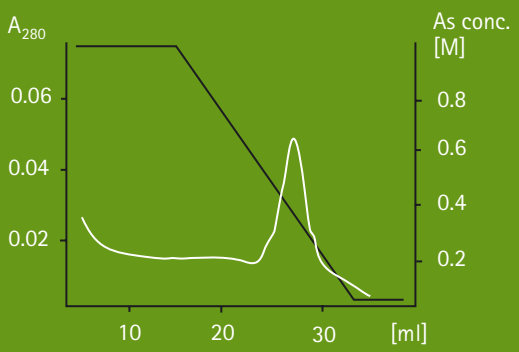



**Fig. 4**
*Recombinant single-chain antibody fragment purified on Fractogel® EMD TA (S) (buffer A: 50 mM sodium phosphate, 1 M ammonium sulphate; pH 8.0; buffer B: 50 mM sodium phosphate; pH 8; flow rate: 0.8 ml/min)*

## Scaling-up

Fractogel® EMD tentacle media are delivered as bulk material, packing protocols are available on request. The most commonly used columns for HIC have diameters of 10 to 300 mm and the bed height should be between 10 and 30 cm.

## Regeneration

Regeneration and how often it is necessary depends on the sample. A typical regeneration procedure consists of washing the column with an alkaline solution (0.1-0.5 M NaOH) and reequilibrating the column with the initial buffer solution (or storage buffer). Alternatively, the column can be regenerated by urea (6M), water or organic solvents (ethanol, 2-propanol, ethylene glycol). For example, rinsing with 20% methanol at a flow rate of 0.5-1 cm/min can be recommended.

# Thiophilic adsorption chromatography (TA) on tentacle media

Thiophilic adsorption chromatography is a group-specific, salt-dependent purification technique based on the ability of immunoglobulins to bind to sulfone groups that lie in close proximity to a thioether. Salts that interact with water molecules (e.g. ammonium sulphate) promote this adsorption. Fractogel® EMD TA for thiophilic adsorption chromatography is synthesized by the tentacle technology, where the group specific ligands are present at high density. Thus, the thiophilic tentacle material has a high protein binding capacity and is suitable for purifying antibodies at the analytical as well as the preparative scale. As known from the tentacle-type ion exchangers, the linear polymer chains provide an appropriate spacing which results in minimized non-specific interactions with proteins combined with high protein binding capacities. The “3 S-type” heteroaliphatic ligand, present in Fractogel® EMD TA, shows significantly increased thiophilic interactions compared to “2 S-type” ligands which seem to resemble more closely solely hydrophobic materials **(Fig. 3)**.

The binding of the protein takes place mainly via accessible tryptophane and/or phenylalanine residues.

## Application area

TA is very useful for the purification of immunoglobulins (monoclonal and polyclonal antibodies). Albumins are not adsorbed on thiophilic media, which often simplifies the effective separation of antibodies. Antibodies of the IgM sub-class can also be bound on a Fractogel® EMD TA column. The binding of antibodies from different species to thiophilic adsorption supports offers advantages when compared to the Protein A method. In particular, elution conditions at physiological pH values, enables high recoveries of biologically active antibodies without having to neutralize the eluate. All antibodies tested so far bind to Fractogel® EMD TA, including Fab Fragments and recombinant single-chain antibody fragments **(Fig. 4)**.

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Merck KGaA
Life Science & Analytics
64271 Darmstadt, Germany
Fax +49 (0) 61 51/72 - 91 66 67
E-mail: processing@merck.de
Internet: LSA.merck.de