Data sheet

MBP SepFast
MBP SepFast HighRes

MBP SepFast is a chromatography medium for purifying recombinant proteins tagged with maltose binding protein (MBP). It is a scalable medium.

Tagging proteins with MBP often gives increased expression levels and higher solubility of the target protein. Proper folding of the attached protein has also been shown to be promoted by the MBP tag. With the benefit of increased solubility, the tag could be particularly useful for recombinant proteins expressed in an insoluble form (inclusion bodies).

Affinity purification using MBP SepFast takes place under physiological conditions and mild elution is performed using maltose. This preserves the activity of the target protein. Even intact protein complexes may be purified. In addition, the high specificity of the binding means that very high purity can be achieved in just one step in combination with high binding capacity.

MBP SepFast is a robust, high resolution medium based on the well known agarose matrix. The small and evenly sized beads ensure that MBP-tagged proteins elute in narrow peaks, thus minimizing the need for further concentration steps. MBP SepFast tolerates all commonly used aqueous buffers and is easily regenerated using 0.5 M NaOH allowing the same medium to be used for repeated purifications.

The base matrix is made of highly cross-linked porous agarose. The particle has open pore structure with good mass transfer property to large protein molecules. The medium shows high mechanical rigidity. So it can be operated at high flow velocities with moderate pressure drop.

Table 1: Characteristics of MBP Resins:

Matrix	Cross-linked agarose
Functional group	Dextrin
Particle size	50 - 150 μm (MBP SepFast) 20 - 50 μm (MBP SepFast HighRes)
Binding capacity	Protein dependent
pH stability	2-13 (short term) and >7 (working range)
Working temperature	+4^{O}C to +30^{O}C
Chemical stability	All commonly used buffers, 6M guanidine-HCl, 8 M urea, 0.5 M NaOH (for regeneration and cleaning)
Storage	Store in 20% ethanol

Method optimisation

The commonly used methods for MBP protein purifications can be directly adopted to this resin. The common buffers can be tris or phosphates at pH >7. Salts, EDTA or DTT can be added to adjust the binding conditions or to stabilise the target proteins. 10 mM to 50 mM maltose in the binding buffer is normally employed to elute the MBP.

The presence of reducing agents, e.g., 5 mM DTT, may decrease yield. Higher ionic strength does not decrease affinity since MBP binds to the resin primarily by hydrogen binding. Agents that interfere with hydrogen binding, such as urea and guanidine hydrochloride,

are not recommended. The presence of 10% glycerol may decrease the yield and 0.1% SDS completely eliminates the binding

In general, balancing product recovery against process throughput is the major consideration when optimizing a method.

Regeneration

After each run, elute any reversibly bound material with 0.1 to 0.5 M sodium hydroxide.

Cleaning-in-place (CIP)

CIP is a procedure that remove strongly bound materials such as lipids, endotoxins and denatured proteins that remain in the column after regeneration. Regular CIP prevents the build up of contaminants in the packed bed and helps to maintain the column performance.

A specific CIP protocol should be developed for each process according to the type of contaminants present. The frequency of CIP depends on the nature of individual applications.

The following information works as a general guidance.

The contaminants bound by hydrophobic nature can be removed by the following reagents: 0.5 M NaOH, low percentage non-ionic detergents (e.g. 0.1 – 2%), 30% isopropanol in basic or acidic conditions (e.g. in the presence of acetic acid or phosphoric acid). A combination of the above reagents can be explored as well. In general, the incubation time should be longer (e.g. from 30 minutes to 2 hours) to ensure full dissociation of the contaminants. Long contact time should be avoided when alcohols are used, as the acrylic column body may be damaged.

Sanitization

Sanitization using 0.1 -0.5 M NaOH with a contact time of 1 hour is recommended.

Storage

The resin should be stored in 20% ethanol to prevent microbial growth. Store the resin at a temperature of +4^{o}C to +30^{o}C. After storage, equilibrate the resin with at least 5-bed volumes of the binding buffer before use.

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