Data sheet

Protein A SepFast™ MAG

Protein A SepFast MAG is specially designed and fabricated for magnetic purification of immunoglobulins in batch mode (i.e. stirred tank mode). The base matrix is made of cross-linked polysaccharide encapsulating magnetic material. Therefore, the resin possesses magnetic properties. Removal of liquid after each step such as binding, washing and elution can be readily done by fixing the resin with a magnet.

This product can be used with any magnetic device commonly available in the market place or magnets of any shape.

Protein A binds to the Fc region of immunoglobulins. The binding is highly specific so high purity can be achieved in a single step. The purification power of Protein-A ligand has been well documented in various antibody purification applications, such as isolation and purification of classes, subclasses and fragments of immunoglobulins from biological fluids and from cell culture media.

1. Properties

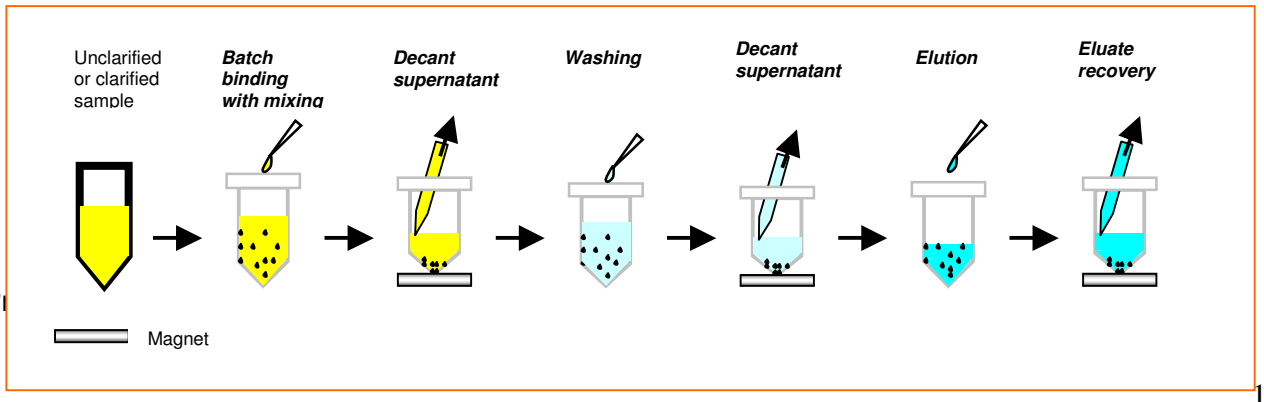
Protein A is immobilised to a highly porous and highly cross-linked agarose base matrix. Agarose has long been used for chromatographic separations due to its excellent hydrophilic and low non-specific-binding nature. The particles have an open pore structure with excellent mass transfer properties to large protein molecules. The medium shows high mechanical rigidity.

Protein A SepFast MAG is highly stable and compatible with a wide range of chemicals commonly required in antibody purification processes.

Table 1: Characteristics of Protein A SepFast MAG:

Matrix	Cross-linked agarose encapsulating magnetic material
Binding capacity	Approx. 15 mg human IgG / ml
pH stability	2-10 (short term) and 5-9 (long term)
Working temperature	+4^{O}C to +30^{O}C
Chemical stability	Compatible with most commonly used reagents for antibody purifications
Sanitisation	Wash the resin with 2% hibitane/20% ethanol
Storage	20% ethanol at +4^{O}C - +8^{O}C

2. General operations



1. Fully shake the bottle to re-slurry the resin. Take out the required amount of the slurry using a 200 μl pipette (Note: the pipette tip should be cut off approx. 3-5 mm from the narrow side to avoid possible tip blockage by the resin particles) and transfer to a suitable container (e.g. an eppendorf centrifuge tube) for batch binding.

2. Wash the resin with 5 - 10 resin volumes of the equilibration / binding buffer. After settling the particles using a magnet, remove the supernatant using a pipette. Repeat the washing two more times. Be sure the magnet is removed when the washing buffer is added and mixed.

3. After washing step, add the protein sample. Close the container lid and place in a suitable mixer for batch binding. Depending on the nature and size of the target protein, the binding time varies from a few minutes to up to a few hours. Generally speaking, 10 – 30 minutes is sufficient to utilise most of the resin capacity.

4. After the batch binding is finished, drag down the resin with a magnet. Remove the supernatant using a pipette. Depending on the strength of the magnet used, care should be paid not to suck the magnetic particles away. Wash the resin three times (2 – 3 resin volumse each time) with the washing buffer. The stringency of washing solution may vary each time to remove as much impurity as possible. Collect the waste in a suitable container.

5. Elution can be done in batch incubation. Generally speaking, the eluent volume at 3 – 5 times the resin volume is sufficient to recover the bound protein. For the best recovery yield, incubation of the resin-eluent mixture in a roller mixer for 5 - 10 minutes is recommended. This allows sufficient time for the internally bound protein molecules to diffuse out of the macropores. The eluate is recovered through pipetting after the particles are dragged down with a magnet. This step may be repeated once or twice to maximise the recovery yield.

3. General considerations

3.1 Binding

Protein A SepFast MAG binds IgG from most species at neutral pH (e.g. pH 7 to 7.4) and physiological ionic strength (e.g. phosphate saline buffer). The static binding capacity depends on the source of the particular immunoglobulin.

3.2 Elution

The bound immunoglobulin is normally eluted by reduced pH, such as about pH 3.0. The general elution buffer includes 0.1M glycine pH 3.0 or 0.1M citric acid pH 3.0. For very strongly bound molecules, the pH may reduce to between 2 to 3.

For acid labile proteins, the eluted fractions can be quickly neutralized by adding (or pre-added) 1M Tris/HCl, pH 9.0 (10% to 20% v/v).

3.3 Regeneration

After the elution, wash the medium with 2 – 3 volumes of the elution buffer following with 3 – 5 volumes of the equilibration buffer.

4. Cleaning-in-place (CIP)

In some applications, substances such as denatured proteins or lipids stay in the resin after the regeneration step. The following cleaning procedure could be carried out.

To remove precipitated or denatured materials, wash the resin with 2 column volumes of 6 M guanidine hydrochloride followed immediately with at least 5 column volumes of the binding buffer. To remove the bound hydrophobic components, wash the resin with 1 column volume of a non-ionic detergent e.g. 0.1% Triton™ X-100 at 37°C followed immediately with at least 5 column volumes of the binding buffer.

5. Sanitization

Equilibrate the resin with a buffer containing 2% hibitane gluconate and 20% ethanol. Allow to stand for 6 to 8 hours. Re-equilibrate the resin with at least 5 column volumes of sterile binding buffer.

6. Storage

Store the loose medium in the presence of 20% ethanol at 4-8°C. Never freeze the medium.

7. Further information

Visit www.biotoolomics.com for further information or contact the technical team or sales representatives.

8. Ordering information

Product	Quantity	Code no.
Protein A SepFast MAG	500 µl	280101
	5 ml	280102

BioToolomics Ltd
Unit 30A, Number 1 Industrial Estate
Consett
County Durham, DH8 6TJ
United Kingdom

www.biotoolomics.com