BIOTOOLOMICS

Glutathione SepFast MAG Media

Data and Instructions

1. Introduction

Glutathione SepFast MAG is a magnetic affinity chromatography medium used for the rapid one-step purification of Glutathione S-transferase (GST)-tagged proteins with the aid of a magnet. Other Glutathione S-transferases and Glutathione-binding proteins can also be purified with this adsorbent.

Reduced Glutathione ligand is immobilised to the carefully designed magnetic support via very stable thiolether linkages.

GST-tagged proteins expressed in bacteria, yeasts, insects and mammalian cell cultures can be readily purified in a single purification step. After binding of target molecules, the magnetic resin can be readily isolated from the biological feedstocks with the aid of a magnet. GST tag can be cleaved in a bound condition or in an eluted condition by specific proteases.

2. Product characteristics

SepFast MAG is specially designed and fabricated for magnetic purification of proteins in batch mode (i.e. stirred tank mode). The base matrix is made of cross-linked agarose containing 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.

Note: no magnet is supplied with this product. Most of the commercial magnetic devices can be used to handle this resin.

Its carefully controlled pore structure allows fast accessto affinity ligands by target protein molecules. Also, its high mechanical strength permits harsher operational conditions. Clarified or unclarified cell lysates (for intracellular proteins) or culture broths (for extracellular proteins) could be directly processed with Glutathione SepFast MAG. All these translate into high process flexibility and higher protein yield at shortened purification time.

Table 1. Product characteristics

Particle size	50 – 150 μm
Base matrix	Highly cross-linked magnetic agarose
Ligand	Glutathione
Ligand density	≥ 20 μmol / ml resin
Protein binding capacity	Depends on the type of proteins and binding conditions; could be > 10 mg / ml resin
Chemical stability	Compatible with all the commonly used aqueous buffers; stable at short contact to denaturants (e.g. 6M guanidine.HCl or 8M urea); stable to common clean-in-place agents e.g. 70% ethanol, 0.1 M NaOH, 0.1 M HCl.
pH stability	4-12
Storage	20% ethanol at 4°C – 8°C

Glutathione SepFast adsorbent is highly stable and compatible with a wide range of chemicals commonly required in protein purification processes, which means that more flexible operations can be developed for the best performance.

3. Purification procedures

Please read Section 4 before a purification experiment is designed.

Target protein can be directly purified from unclarified or clarified cell lysates no matter if it is batch or gravity operated. As GLUTATHIONE SepFast MAG is compatible with most of the commonly used reagents in biological systems, cell lysates generated by the commercial cell lysing reagents / kits etc in the market place can be directly used with GLUTATHIONE SepFast MAG without extra treatment (note: for precaution it is recommended to test in small scale first). Recombinant proteins expressed as inclusion bodies can be directly purified (and refolded if necessary) after dissolving in denaturing reagents e.g. 6 M GuHCl or 8 M urea.

3.1 Preparations before protein purification

Protein expression and cell culturing are referred to in the well-established protocols in the literature.

Harvest the cells and / or broth after the culture is finished. For intracellularly expressed proteins, the pelleted cells are generally resuspended in PBS or other suitable buffers for following cell disruption such as freeze-thaw, ultrasonication, homogenisation and bead milling etc. Or the pelleted cells can be directly suspended into a self-made or commercial cell lysing solution for the release of the target protein (refer to the well established protocols in the literature). Proteins expressed as inclusion bodies can be dissolved in denaturing reagents such as GuHCl and urea first. Clarified or unclarified protein samples can be purified directly. If an unclarified sample is loaded, a treatment with DNAse I (e.g. 5 μg/ml of Benzonase with 1 mM Mg^{2+} for 10-15 mins in ice-bath) may be required to reduce the sample viscosity. Pre-conditioning of the cell lysates, such as pH adjustment, can be done in this step.

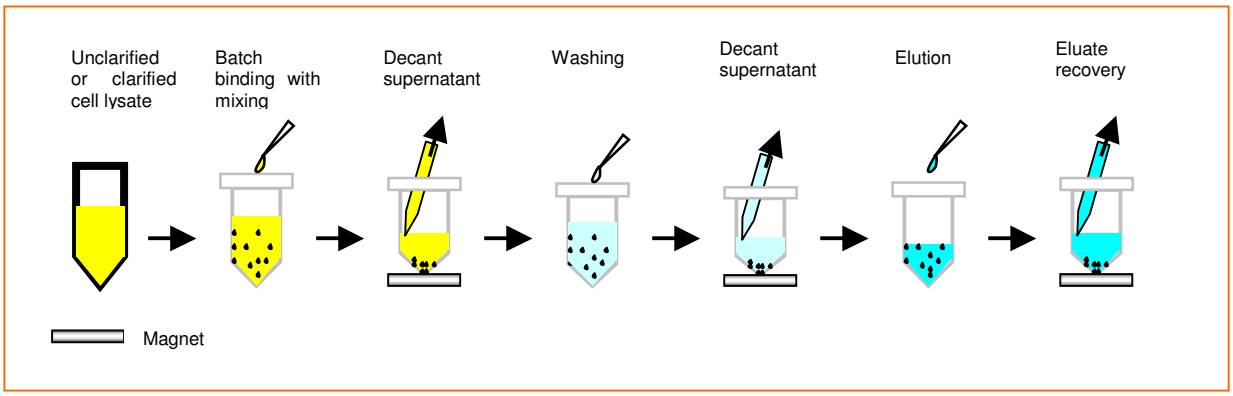
Equilibration / binding buffer is recommended as: PBS, pH 7.4.

Washing buffer can be the same as the binding buffer or may contain additional reagents (e.g. detergents, alcohol etc) or have low pH value etc, in order to remove as much weakly bound impurities as possible.

Elution solution is recommended as: 50 mM Tris / HCl containing 10 mM reduced glutathione, pH 8.0.

Water and chemicals used for the protein purification process should be of high purity.

3.2 Protein purification procedure



1. Depending on the quantity of target protein in the cell lysate, the amount of GLUTATHIONE SepFast MAG is estimated at a binding capacity of 5 mg/ml resin (the actual capacity could be higher). This figure is divided by 0.5 to give the volume of resin slurry required, as the resin is supplied at a 50% v/v concentration. For example, 10 ml of cell lysate contains 5 mg of GST-tagged protein. So 1 ml of the resin is enough, which translates into a slurry volume of 2 ml.

2. 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. a eppendorf centrifuge tube) for batch binding.

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

4. After washing step, add the protein sample in. Close the container lid and place onto a suitable roller mixer for batch binding. Depending on the nature and size of 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.

5. 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 (3 – 5 resin volumes each time) with the washing buffer. The stringency of washing solution may vary each time to remove as many impurities as possible. Collect the waste in a suitable container.

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

7. Buffer exchange and / or desalting might be required to adjust the pH and to remove glutathione in the eluted sample. SuperSpin Desaltor (product code: 210101) can be used as a fast and cost-effective approach. Refer to the product files (www.biotoolomics.com/product) for more details.

4. Clean-in-place (CIP)

In the case that the resin is severely contaminated by strong ionic substances (e.g. proteins and nucleic acids etc), hydrophobic proteins, lipoproteins and lipids etc, the following clean-in-place approaches can be conducted.

Salt with concentration up to 2 M can be used to clean the impurities bound by ionic interactions.

The contaminants bound by hydrophobic nature can be removed by the following reagents: low percentage detergents (e.g. 0.1 – 2%), 70% ethanol or 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 overnight) to ensure full dissociation of the contaminants.

5. Trouble shooting

The following tips may help to resolve the possible problems with individual purification process. If you would further assistance, please contact our technical team or sales representatives for more information.

No or little target protein in the eluted fractions	1. **Elution condition is too mild to dissociate the bound protein**. Increasing the glutathione concentration or the pH in the elution buffer may help. 2. **Binding conditions are not correct**. Check pH and composition of all buffers and solutions in each step. 3. **Binding time or elution time is too short**. Try to increase the contact time between resin and the buffer in each step. 4. **GST tag is not present**. Check the protein gene construction is correct and as it was originally designed. 5. **GST-tag has been degraded**. Use anti-GST antibodies in western blotting to check the location of the tag. Lysate preparation step needs be improved. 6. **GST tag is not sufficiently accessible**. Denaturing reagents such as urea could be added to partially defold the protein. 7. **The target protein has precipitated on the resin**. Try to add detergents (e.g. 0.1 – 1 % v/v) or denaturing reagents (e.g. 4 – 8 M urea). 8. **Add DTT prior to cell lysis and to buffers:** Adding DTT to a final concentration of 1-10 mM may significantly increase binding of some GST-tagged proteins.
The target protein is eluted with impurities	1. **Binding and washing conditions not stringent enough**. Washing with larger volumes or longer time can be tried. 2. **Impurities are associated with the target protein**. Try to add reducing reagents (e.g. <10 mM β-mercaptoethanol) in the sample or washing buffer to disrupt formation of disulfide bonds. Try to add detergents or alcohol / glycerol in the washing buffer to suppress any non-specific interactions. 3. **Impurities are truncated parts of the target protein**. Check the gene construction and expression conditions to minimise potential mutations. Prevent protein degradation by addition of protease inhibitors and / or reduction of working temperature. 4. **Impurities have higher affinity than the target protein**. Careful optimisation of the binding, washing and elution conditions might allow the recovery of target protein without co-elution of the tightly bound impurities. For example, milder elution conditions may help.

6. Storage

Store the resin in 20% ethanol at 4°C to 8°C. Seal the bottle soon after each use.

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.
Glutathione SepFast MAG	1 ml	320201
	5 ml	320202
	25 ml	320203
Related products		
SuperSpin Desaltor	50	210101

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