BIOTOOLOMICS

Glutathione SepFast Media

Data and Instructions

1. Introduction

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

Reduced Glutathione ligand is immobilised to the carefully designed porous 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 step. The GST tag can be cleaved in a bound condition or in an eluted condition by specific proteases.

2. Product characteristics

Glutathione SepFast adsorbents are specifically designed and fabricated for the purification of GST-tagged proteins in batch (stirred tank), gravity flow or packed column modes. Glutathione SepFast is made of highly cross-linked agarose beads. Its carefully controlled pore structure allows fast access to affinity ligands by target protein molecules. Also, its high mechanical strength permits liquid to pass through gravity columns or packed columns at excellent flow rates. Clarified or unclarified cell lysates (for intracellular proteins), or culture broths (for extracellular proteins) could be directly processed with Glutathione SepFast. 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 4% 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); compatible with common clean-in-place agents e.g. 70% ethanol, 0.1 M NaOH, 0.1 M HCl.
pH stability	3-12
Storage	20% ethanol at 4°C – 8°C

Glutathione SepFast BG is supplied as a loose resin or is supplied as pre-packed columns.

To facilitate the purification process with as little hassle as possible in batch or gravity flow operations, two specially designed plastic columns can be ordered separately to handle the resins. The larger column BG-30 (product code: 200101 and 200102) has a working volume of 30 ml with volumetric marks in the first 20 ml (at 1 ml interval). The smaller one BG-5 (product code: 200103 and 200104) has a working volume of up to 5 ml. There is a sintered porous mesh underneath each column to withhold chromatography particles. Both lids in the bottom and the top of the column are provided. Therefore, the whole protein purification process, i.e. resin equilibration, binding, washing, elution and regeneration (or clean-in-place) can be done within the same column in a very economic way. For further process flexibility, the exact volume of required resin can be loaded to the column of choice without limitation. In comparison, other prepacked gravity columns in the market place have fixed resin volumes (e.g. typically 0.1 ml or 1 ml). The smaller column is ideal for working resin volume of 0.1ml to 1 ml. The larger one is ideal for resin volume of 1 ml to 10 ml.

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 proteins can be directly purified from unclarified or clarified cell lysates no matter if it is batch or gravity operated. As GLUTATHIONE SepFast 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 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 Choice of the operation modes
The ***purification mode***, i.e. batch adsorption, gravity column adsorption, purification with magnetic resin, centrifugal adsorption or packed column adsorption etc, can be selected according to the guidance set in the ***Section 4.2***.

3.2 Preparations before protein purification
Protein expression and cell culturing are referred to in the well established protocols in 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 releasing of the target protein (refer to the well established protocols in literatures). 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.

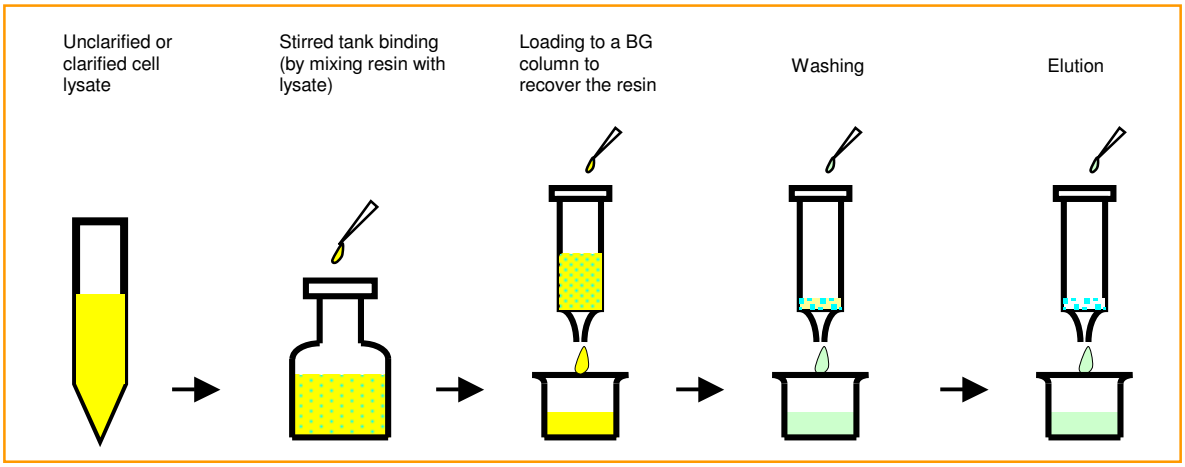
Equilibration / binding buffer is recommended as: PBS, pH 7.4.

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 many 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.

3.3 Protein purification in a batch stirred tank mode



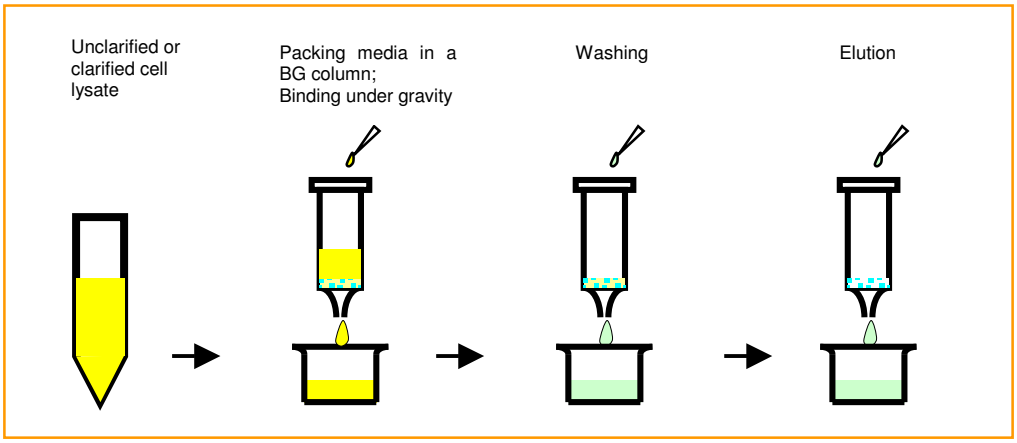
1. Depending on the quantity of target protein in the cell lysate, the amount of GLUTATHIONE SepFast is estimated at a binding capacity of 5 mg/ml resin (the actual capacity could be higher). This figure is divided by 0.5 to give the volume of resin slurry required, as the resin is supplied at a 50% 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 resin bottle to re-slurry the resin. Take out the required volume of the slurry using a 1.0 ml pipette (Note: the 1.0 ml 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 the BG-30 or BG-5 column or other suitable containers. (Note: shake the bottle to reslurry the resin immediately before pipetting to avoid settling. Seal the bottle and store at 4°C soon after the resin is taken out.)
3. Hold the column in a vertical position using a proper stand. Wash the resin with at least 10 resin volumes of the equilibration buffer under gravity until the liquid is fully passed through. Note: gently tap the sinter side of the column if no liquid comes out.
4. If the volume of protein sample is less than the working volume of the column, put on the bottom lid and load the protein sample in to the column. Otherwise go to step 5. Close the top lid and place the column on a suitable roller mixer for batch binding. Be sure the resin is fully mixed with the protein sample. 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.
5. If the volume of protein sample is beyond the working volume of the column, re-slurry the resin with a few mls of the binding buffer and pour it into a suitable bottle in which, the protein sample is loaded for batch stirred tank binding. Place the bottle on a proper roller mixer. 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.
6. After the batch binding is finished, place the column in a suitable stand in a vertical position. Remove the top lid then the bottom lid. In case of the sample being processed via Step 5, the slurry is poured into the column to recover the resin. The cell lysate (clarified or unclarified) is discharged by gravity or by suction through a syringe (connecting the bottom side of the column to a syringe via rubber tubing). It is recommended to use this suction approach, as it is a much quicker way of removing the liquid.

7. Put the bottom lid on, add a few mls of washing solution. Shake to mix the resin with liquid for 10-30 seconds. Remove the liquid by gravity discharge or by syringe suction. Repeat the washing 2 or 3 times. The total washing volume should be at least 10 resin volumes. Collect the waste in a suitable container.

8. Elution can be done in gravity flow mode or by batch incubation. Generally speaking, the total volume of elution buffer at 5 – 10 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 under gravity flow or by syringe suction, and collected in a suitable container. This step may be repeated once or twice to maximise the recovery yield.

9. 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.

3.4 Protein purification in gravity flow mode



1. Depending on the quantity of target protein in the cell lysate, the amount of GLUTATHIONE SepFast 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. The small chamber under the sintered mesh of the BG column of choice has to be pre-filled with water and ***should be air bubble free*** (otherwise, the resin may have reduced binding capacity). To do so, fill the column full with water. Remove the bottom lid to let a few drops of liquid come out. If no liquid comes out, tap the column on working benchtop a few times. If an air bubble is trapped underneath the sintered mesh, close the top lid and then turn the column upside down. Tap the mesh side to let the trapped bubble escape. Inverst the column and open the top lid to let liquid through. When the chamber is fully filled with water and is free of air bubble, put the bottom lid on. Pour out the free water above the mesh. The column is ready to accommodate resins.

3. Fully shake the bottle to re-slurry the resin. Take out the required amount of resin, according to the calculation in Step 1, using a 1.0 ml pipette (Note: the 1.0 ml pipette tip should be cut off approx. 3-5 mm from the narrow side to avoid possible tip blockage by the resin particles). Transfer it into the column. (Note: Seal the bottle and store at 4^{o}C soon after the resin is taken out.)

4. Hold the column in a vertical position in a proper stand. Remove the bottom lid. Equilibrate the resin by gently adding 5 – 10 resin volumes of the equilibration buffer and discharging under gravity until the liquid has fully passed through.

5. Put the bottom lid on. Load the protein sample into the column in the following manner. Add the first 5 ml of protein sample slowly in a BG-30 column (or 2 ml if a BG-5 column is used) using a pipette to minimise the disturbing of the settled particles. The rest of the protein sample is then gently poured in. If the protein sample is over the working volume of the column, fill the column full first. Remove the bottom lid to let the feedstock pass through under gravity. The protein sample can be further poured in when more column space is available. Collect the flow through in a suitable container for future analysis.

6. After the above binding process is finished (i.e. the whole protein sample passes through the column), washing solution is gently added to remove the weakly bound impurities. At least 5 bed volumes of washing liquid is required. Collect the washing waste in a suitable container for further analysis.

7. Load the elution buffer in the column in a way that is as gentle as possible to avoid disturbing the settled particles. Collect the eluate in a suitable container. The elution may be repeated once or twice to maximise the recovery yield. The total eluant volume at 5 – 10 times the resin volume is sufficient to recover the bound protein.

8. Buffer exchange and / or desalting might be required to adjust the pH and to remove glutathione in the eluted sample. Depending on the sample volume, SuperSpin Desaltor (product code: 210101) can be used for

fast and cost-effective desalting /buffer exchange. Refer to the product files (www.biotoolomics.com/product) for more details.

3.5 Protein purification in packed bed mode

1. Set up the chromatography system. Check the tubings and pumps work in right order.
2. Pack the required amount of resin into the column of right size (refer to established protocols or the column supplier's guidance).
3. Ensure the resin has been packed properly (e.g. no air bubbles trapped somewhere inside the column).
4. Apply 3 – 5 column volumes of distilled water at a flowrate of 250 cm/h to remove any ethanol. If the media is compressed with a liquid gap above, carefully adjust the column plunge to let the mesh be in contact with the media.
5. Equilibrate the resin with 5 – 10 column volumess of the binding buffer at a flowrate of 150 cm/h. The flowrate can be optimised depending on the nature of the protein. It should always be remembered that the operational flowrate is less than the packing flowrate (e.g. at 75% or so).
6. Load the right sample to the column at the same flowrate as above.
7. Wash the column with the binding buffer until it reaches the baseline.
8. Further wash the column with the washing buffer (if different from the binding buffer) at 3 – 5 column volumes.
9. Elute the bound protein with the elution buffer stepwise or in a linear gradient, at the flowrate the same as above or lower. Collect the elution fractions for further analysis.
10. 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. Choice of operational modes

The following operational modes are suitable for consideration: batch stirred tank mode (including magnetic operation with magnetic particles), gravity flow mode and pressurised (packed) column mode (operated with an instrument or manually by syringe).

For laboratories that have no access to expensive and sophisticated process chromatography systems (e.g. packing columns, pumps and valves, detectors and associated computing machinery etc), or to whom protein purification is only a tool rather than their core research activities, the best choice is among batch stirred tank mode, gravity flow mode and packed column-syringe mode.

Batch stirred tank operation is the most versatile and flexible approach. Very little training in chromatography is required. It is particularly attractive in cases when the volume of protein sample is large but the concentration is relatively low or the target protein molecule is large (e.g. over 65K Daltons). Longer contact time can be employed in this mode to allow protein molecules to diffuse into the pores, which means better utilisation of the whole resin ligands and subsequently higher binding capacity. Also, there is no limitation from the column. So, large volumes of protein sample can be processed in the same time period as that for small volumes. GLUTATHIONE SepFast is particularly suitable for this operation. As specially designed columns (BG-30 and BG-5) are supplied to facilitate the separation between liquid and the solid resin particles, the hassle associated with the conventional batch operation, such as slow gravity settling of resin, centrifugal settling of resin and decanting of liquid (with potential loss of resin), have been removed.

Magnetic GLUTATHIONE resin such as GLUTATHIONE SepFast MAG is very suitable to batch stirred tank operation, particularly when the volume of protein sample and the quantity of required resin is small. Solid / liquid separation in each step (e.g. equilibration, binding, washing and elution etc) is readily achieved by use of a magnet such as a magnetic stirring bar.

Gravity flow operation is a good choice if the volume of protein sample is from a few mls to tens of mls, and the protein molecule isn't very big. More than one column can be easily operated in parallel. GLUTATHIONE SepFast resins possess strong mechanical structure to permit liquid to pass through at an excellent flowrate. The particle size and pore structure are carefully controlled for such operations. The specially designed BG-30 and BG-5 columns are ideal for such operations.

Pressurised (packed) column operation gives the best chromatographic performance. GLUTATHIONE SepFast is particularly designed for such operations. However, expensive and specialised instruments and accessories are required for this kind of operation. Professional training and expertise in process chromatography is essential in order to benefit from such operations.

5. 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.

6. Trouble shooting

The following tips may help to resolve the possible problems with individual purification process. If you would like to get 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 a larger volume or for a 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 interaction. 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.
Column is clogged	1. **The unclarified protein sample is too viscous**. Treatment by ultrasonication or addition of DNase (e.g. 5 μg / ml benzonase + 1 mg / ml of Mg^{2+} in ice-bath for 10-15 mins) can reduce the viscosity. 2. **Air bubble is trapped under the sintered mesh**. Tilt the column (or even fully invert the column) and tap the mesh side to let the bubble escape. 3. **Column mesh is blocked**. Replace with a new column.

7. Storage

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

8. Further information

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

9. Ordering information

Product	Quantity	Code no.
Glutathione SepFast	5 ml	320101
	10 ml	320102
	50 ml	320103
	1 litre	320104
Pre-packed Glutathione SepFast column (disposable)	1 x 1 ml	320105
	1 x 2.5 ml	320106
	1 x 5 ml	320107
	1 x 10 ml	320108
	1 x 20 ml	320109
Related products		
BG-30 column	20	200101
	50	200102

BG-5 column	20	200103
	50	200104
SuperSpin Desaltor	50	210101

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