USER GUIDE: AM-50 Glutathione resin

This product consists of glutathione attached through the sulfur to epoxy activated polyacrylate resulting in a 12-atoms spacer and is suitable for affinity purification of GST-fusion proteins and other S-tranferases or glutathione-dependent proteins. EBL AM-50 Glutathione affinity chromatography permits rapid, mild, non-denaturing, and highly selective purification of glutathione-dependent proteins.

EBL AM-50 series were polyacrylate-based medium with high binding capacity, high performance and high flow rate, providing extraordinary flow characteristic, excellent for scaling-up.

Specifications:

Particle Size (µm)	50
Pore Size (Å)	800
Ligand Density	150-350 µmol GSH/ ml medium
Target Protein Binding Capacity (g/ml)	≈10 mg recombinant glutathione
Bead Matrix	Polyacrylate, PS/DVB
Recommended Flow Rate (cm/h)	150-700
pH Stability	3-13
Pressure (MPa)	10
Storage buffer	20% ethanol
Storage temperature	+4–30 °C

Operation instruction:

Buffer preparation

Filter the buffers by a 0.22 um or 0.45 um filter before use. The AM-50 Glutathione resin must be washed thoroughly with 10 times volume of water or Equilibration Buffer (such as PBS) to remove the ethanol.

1. **Binding buffer:**

PBS (140 mM NaCl, 2.7 mM KCl, 10 mM Na2HPO4, 1.8 mM KH2PO4, pH 7.3). Dissolve moderate amount of protease inhibitors during cell lysate preparation.

PBS-T (PBS with 1% TRITON X-100)

2. **Elution Buffer:**

10 mM reduced glutathione in 50 mM Tris-HCl, strorage condition: pH 9.5 at 4 °C or pH 9.0 at 25 °C

Note: 1-10 mM DTT within the binding and elution buffers can be increase purity, but may result in lower yield of target protein.

Procedure:

AM-50 Glutathione resin can be applied in columns or for batch purifications. Also consider this procedure as a guideline for column purifications. For batch purifications, apply low revolution centrifugation (200-500 g for 10s) to allow the beads to form pellets and decant between every step.

- **Column Setup:**

1. Prepare the resin as described in Buffer Preparation.
2. De-aerate the resin and carefully pour the slurry into the column in a manner of not generating air bubbles.
3. After packing the resin in the columns, equilibrate the resin with Equilibration Buffer for a number times of column volume. Do not left the columns dry in any case.

- **Purification:**

1. Prepare cell lysate with moderate amount of buffer.
   Notes: Tris or phosphate buffers, pH 6.5-9.5, are typical lysis buffers compatible with glutathione affinity chromatography.

2. Dissolve TRITON X-100 for a final concentration of 1 % (v/v).
3. Centrifuge the solution with 10,000 g at 4 °C for 10 mins or filter it by a 0.45 µm filter to filter the cell lysate.
   Note: Use only clarified supernatant. To prevent clogging the column, highly viscous samples containing chromosomal DNA or RNA should be sonicated or treated with nuclease to reduce the viscosity, and cellular debris and particulate matter must be removed by centrifugation or filtration.
4. Load the filtered supernatant into the column and allow it to pass through. For batch purification, load AM-50 Glutathione resin and Equilibration buffer in 1:1 ratio into appropriate amount. Incubate sample and agitate it gentlely at 4 °C for 5-30 mins. *Note: Depending on the sample and the flow rate, not all the protein may bind. Multiple passes over the column or closing the loaded column and incubating it on a rotator may improve the binding efficiency.*
5. Wash resin four times with PBS-T at 4 °C.
6. Elute the GST off the resin using 1 ml Elution Buffer for 3 times. For batch purification, mix elution outputs for each step gently for 2 minutes.
7. Analyze each fraction with SDS-PAGE.
8. Free glutathione can be removed from sample by dialysis against chosen buffer .

※ Salt concentrations up to 1 M do not interfere with binding.

※ Typically, lysis buffers contain the Protease inhibitors such as EDTA or PMSF as supplements. Serine protease inhibitors dissolving in the lysis buffers do not interfere with subsequent thrombin or factor Xa treatment for being removed before the proteolysis step.

※ The binding of GST and AM-50 Glutathione resin works in the case of 1 % TRITON X-100, 1 % CTAB, 1 % TWEEN -20, 10 mM DTT, or 0.03% SDS buffer system.

Column Cleansing:

Wash Buffer 1 –
6 M guanidine hydrochloride
Wash Buffer 2 –
70% ethanol

1. Wash the columns with wash Buffer 1 by 2-fold resin volume or wash Buffer 2 by 3-4-fold resin volume.
2. Wash the columns with PBS pH 7.3 by at least 5-fold column volume immediately.
3. Store AM-50 Glutathone resin at +4-28°C in 20% ethanol.
4. Equilibrate with Equilibration Buffer before use.

Important Note:
Reduced glutathione is highly active and unstable in the case of high temperature, pH 6.5 or higher and the presence of oxidants. All solutions with reduced glutathione should be prepared before use and not in the case of high pH, high temperature and the presence of oxidants if possible.