Glutathione Resins

I. List of Components

Store all components at 4°C.

Glutathione-Superflow Resin

Cat. #	Size
8911-1	10 ml
8911-2	100 ml
8911-x	2.5 ml

Glutathione-Uniflow Resin

Cat. #	Size
8912-1	10 ml
8912-2	100 ml
8912-x	2.5 ml

Glutathione S-Transferase (GST) Purification Kit (#K1251-1)

Purchase of the GST Purification Kit provides sufficient reagents for performing five batch/gravity flow purifications of up to 10 mg of GST-tagged protein per column.

- **Five Glutathione-Uniflow Columns**
  Each column is prepacked with 1-ml Glutathione-Uniflow Resin.
- **5 x 100 mg of Glutathione** (reduced)
- **10X Extraction/Loading Buffer** (1.4 mM NaCl; 100 mM Na_2HPO_4; 18 mM KH_2PO_4, pH 7.5):

  To prepare the extraction/loading buffer, dilute 4 ml of 10X Extraction/Loading Buffer with 36 ml of deionized water. If necessary, warm the diluted buffer to room temperature to dissolve precipitated salts, and adjust the pH to 7.5. Prepare fresh.
- **Elution Buffer** (50 mM Tris-Base, pH 8.0):
  Dissolve one vial of 100 mg glutathione (reduced) in 10 ml of the elution buffer and adjust the pH to 8.0, if necessary. Prepare fresh.

II. Additional Materials Required

The following reagents are required but not supplied with the Glutathione-Superflow and -Uniflow Resins:

- **Extraction buffer (loading):**
  140 mM NaCl; 10 mM Na_2HPO_4; 1.8 mM KH_2PO_4 (pH 7.5).
- **Elution buffer:**
  10 mM Glutathione in 50 mM Tris-HCl (pH 8.0). Prepare fresh.
- **Regeneration buffers:**
  Buffer 1: 0.1 M Tris-HCl; 0.5 M NaCl (pH 8.5).
  Buffer 2: 0.1 M Sodium acetate; 0.5 M NaCl (pH 4.5).
  Buffer3: 140 mM NaCl; 10 mM Na_2HPO_4; 1.8 mM KH_2PO_4 (pH 7.5).
- **Alumina** (Sigma, #A-2039)

- **Polypropylene tubes**
- **Centrifuge** (pre-chilled to 4°C)
- **TALON™ 2-ml Disposable Gravity Columns** (#8903-1)
- **Deionized H_2O**
- **Ice**
- **Column** (Pharmacia, HR 10/2 or HR 10/10)
- **Mortar/pestle (for Alumina-based protein extraction)**
- **0.45-µm filter (for FPLC applications)**

III. Batch/Gravity-Flow Purification

A. Preparation of Buffers

Prepare the buffers as specified in Additional Materials (Section II). If you have purchased the GST Purification Kit (#K1251-1), dilute and dissolve the premade buffer solutions as specified in Section I.

B. Preparation of GST-fusion Protein Lysate

Note: Solutions containing GST must be kept at 4°C or on ice at all times.

The method given below is a generic one that is applicable for up to 50 g of *E. coli* cells. Other extraction methods can be used with varying recovery and yield. GST loses its ability to bind glutathione resin when denatured. Do not use strong denaturants such as guanidinium or urea in the purification buffers. Check the Reagent Compatibility Table when designing your purification scheme. The batch/gravity-flow protocol can be used with either Glutathione-Superflow or -Uniflow Resins. However, the FPLC purification protocol (Section IV) is only intended for use with the Glutathione-Superflow Resin.

1. Precool the mortar and pestle, centrifuge, and extraction buffer to 4°C. Place a small polypropylene tube on ice.

2. Transfer cells that express your GST-fusion protein to the precooled mortar. We recommend using 1 ml of resin for every 100–500 mg of cell lysate.

3. Grind 1 part cells with 2.5 parts Alumina for 2–3 min, until the composition of the mixture is paste-like.

4. Add 2 ml of the precooled extraction buffer per 100–500 mg of cells. Centrifuge the cell extract in the precooled centrifuge for 20 min at 10,000–12,000 x g. This procedure will pellet any insoluble material.

5. Carefully transfer the supernatant to the clean, pre-chilled tube. **Do not disturb the pellet.** The supernatant is your clarified sample. If you have purchased the GST Purification Kit, proceed to Section D; otherwise, proceed to Section C.

C. Packing of Glutathione Resin into Disposable Gravity Columns

1. Thoroughly resuspend the Glutathione Resin to achieve a homogenous 50% suspension of resin in the storage solution.

2. Immediately transfer 2 ml of resin suspension to a disposable gravity column (#8903-1). Ensure that the bottom of the column is plugged with a stopper.

3. Allow the resin to settle in the column.

D. Equilibration of Glutathione Resin in the gravity column

1. Remove the stopper and drain the storage solution from the column.
2. Add 4 ml of deionized H_2O to the top of the column and allow it to drain. **Do not disturb the resin.**
3. Repeat (Step 2) three times.
4. Equilibrate the column by adding 4 ml of loading buffer. **Do not disturb the resin.** Allow the buffer to drain.
5. Repeat (Step 4) three times.
6. Replace the column's top and bottom stoppers. Place it on ice to prechill the resin.

E. Batch/gravity-flow Purification of GST-fusion Protein

1. Add 1.5 ml of the clarified GST lysate (Section B.5) to the pre-chilled resin in the column.
   Important: Disperse the resin while you are adding the lysate. To do so, rapidly add the lysate directly to the resin or invert the column a few times after adding the lysate.
2. Place the column upright on ice for 20 min to allow the resin to settle in the column.
3. Remove the column from ice.
4. Discard the top and bottom stoppers and drain the nonadsorbed lysate from the column.
5. Wash the resin by adding 4 ml of pre-chilled extraction buffer to the column. **Do not disturb the resin.**
6. Repeat (Step 5) three times.
7. Elute your GST fusion protein by adding 6 x 1 ml of elution buffer to the column. Collect the eluate in 1-ml fractions on ice.
8. Because glutathione absorbs strongly at 280 nm and masks the absorbance of the eluted protein at low loads, use a Bradford protein assay (Bradford, 1976) as well as SDS-PAGE to identify fractions containing your eluted GST fusion protein.

IV. FPLC Purification using Glutathione-Superflow Resin

PLEASE READ THE ENTIRE PROTOCOL BEFORE BEGINNING

Before starting, prepare buffers as specified in Additional Materials Required (Section II). For the batch/gravity-flow purification protocol, see Section III.

The FPLC protocol cannot be used with Glutathione-Uniflow Resin.

A. Preparation of GST-fusion protein lysate

Note: Solutions containing GST must be kept on ice at all times.

The method given below is a generic one that is applicable for up to 50 g of *E. coli* cells. Other extraction methods can be used with varying recovery and yield.

1. Precool the mortar and pestle, centrifuge, and extraction buffer to 4°C. Place a small polypropylene tube on ice.

2. Transfer cells that express your GST fusion protein to the precooled mortar. We recommend using 1 ml of resin for every 100–500 mg of cell lysate.

3. Grind 1 part cells with 2.5 parts Alumina (Sigma A-2039) for 2–3 min, until the composition of the mixture is paste-like.

4. Add 2 ml of the precooled extraction buffer per 100–500 mg of cells. Centrifuge the cell extract in the precooled centrifuge for 20 min at 10,000–12,000 x g. This procedure will pellet any insoluble material.

5. Carefully transfer the supernatant to the clean pre-chilled tube. **Do not disturb the pellet.** The supernatant is your clarified sample. Proceed to Section B, below.

B. Preparation of Glutathione Resin for FPLC Purification

1. We recommend a column whose internal diameter is at least 1 cm. Columns similar to Pharmacia's HR 10/2 or HR 10/10 are convenient because a volumetric flow rate of 0.78 ml/min can be used during loading. We recommend a bed length of at least 3 cm.

2. Pack the column according to its manufacturer's specifications. We recommend a linear flow rate of at least 5 cm/min for packing. The linear flow rate is the volumetric flow rate, in ml/min, divided by the area of the cross-section of the column (πr^2 where r is the column radius in cm.)

3. Due to the slow binding kinetics of GST to glutathione, a relatively low flow rate must be used during loading. The flow rate for washing and eluting can be increased significantly thus, reducing purification time and increasing yield. At a loading linear flow rate of 1 cm/min, the capacity for GST-fusion proteins from whole cell lysates is approximately 1.5 mg per ml of resin. Equilibration with the extraction/loading buffer can be performed at the same flow rate.

C. FPLC Purification of GST-fusion Protein

1. We recommend that you filter your sample through a 0.45-µm filter before FPLC purification. This action will extend the life of the column.

2. During the loading and washing steps, the linear flow rate should not exceed 1 cm/min; therefore, a column with an internal diameter of 1 cm should not exceed a flow rate of 0.78 ml/min. If fusion protein leakage occurs, the flow rate should be decreased. Once the sample is loaded and the absorbance of the nonadsorbed flowthrough material starts to decrease, you may increase the linear flow rate to 5 cm/min or to 4 ml/min for a column with 1-cm internal diameter. In general, the whole chromatographic purification should not take more than 30–45 min.

3. Elution can be performed at an elevated flow rate, unless the amount of eluted material is much less than the adsorbed material. Collect 1-ml fractions during chromatography and store them on ice.

4. Use a Bradford protein assay (Bradford, 1976) as well as SDS-electrophoresis to identify the fractions containing your eluted GST fusion protein. Western blotting may also be used to identify GST-containing bands with GST Monoclonal Antibody (#3818-1).

V. Regeneration and Storage of Glutathione Resins

Note: If you will not be using the column immediately after regeneration of the resin, complete Steps 1–3, skip Step 4, and proceed directly to Step 5.

1. Wash the column/resin with approximately 10 resin volumes of Regeneration Buffer 1.

2. Wash the column/resin with approximately 10 resin volumes of Regeneration Buffer 2.

3. Repeat Steps 2 and 3 twice.

4. Equilibrate the column/resin with 10 resin volumes of Regeneration Buffer 3.

5. Store resin in a 20% ethanol slurry at 4°C.