User Manual

Glutathione Resin User Manual

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I. Introduction

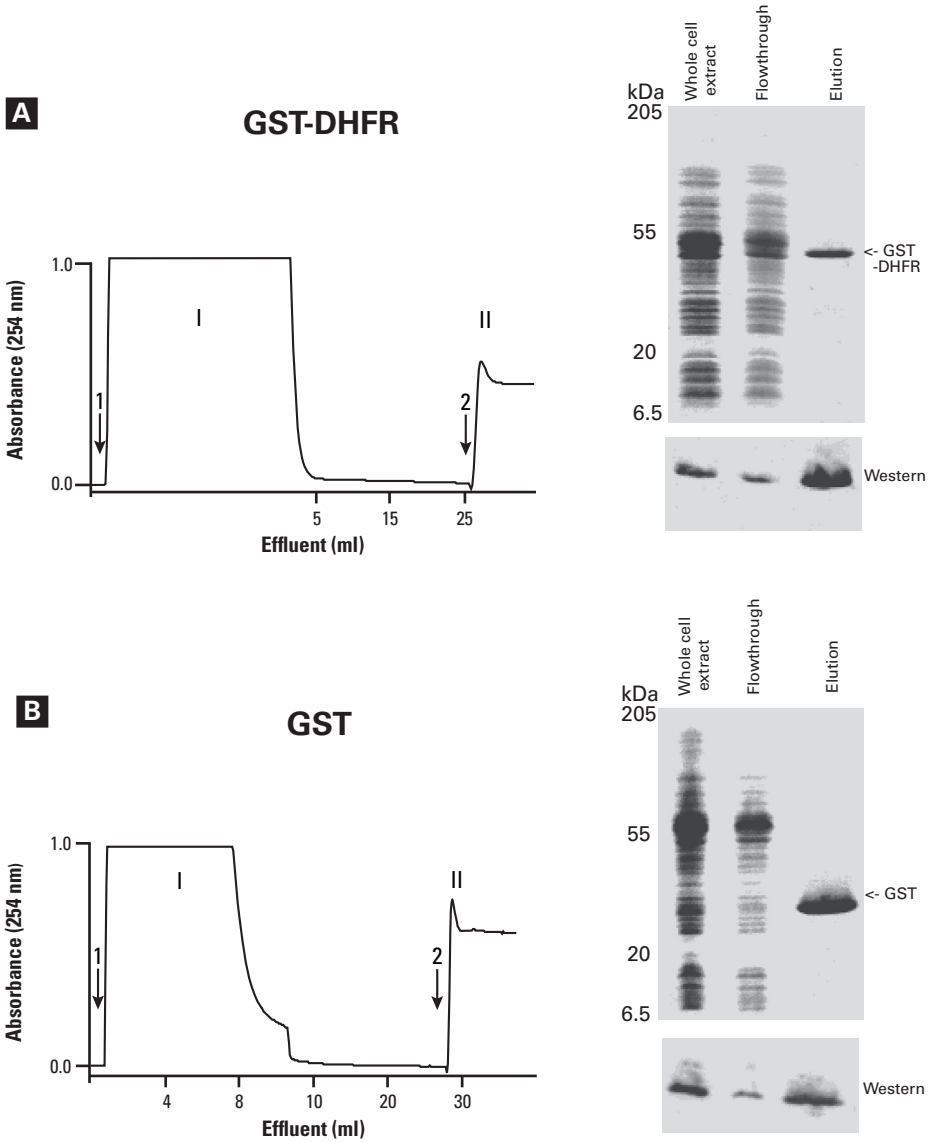
Epitope tags such as GST are often used to label proteins for expression and purification applications. Glutathione transferases are abundant enzymes involved in cellular defense against electrophilic chemical compounds, which bind glutathione with high affinity and specificity. The strength and selectivity of this interaction enables glutathione-based affinity resins to effectively purify GST-tagged proteins. The glutathione resin selectively binds the GST-tagged protein under normal conditions, allowing the one protein of interest to be separated from whole cell extracts rapidly and efficiently. A high degree of purification can be achieved in just one chromatographic step (see Figure).

Glutathione-Superflow Resin allows rapid affinity purification of glutathione S-transferase (GST)-tagged proteins. This resin is based on 6% cross-linked agarose with glutathione covalently bound to the resin. The resin possesses superior structural and flow characteristics for efficient purification of GST-tagged proteins with minimal degradation during processing.

The **Glutathione-Superflow Resin** is suitable for FPLC applications. It can withstand higher flow rates and back pressure with flow rates as high as 15 ml/cm^2/min. It is also suitable for purification of large fusion proteins using batch/gravity-flow purification or standard chromatography applications. For greater convenience, the **GST Purification Kit** provides sufficient stock buffers and prepacked Glutathione-Superflow Columns for performing five batch/gravity-flow purifications. Up to 10 mg of GST-tagged proteins per column can be purified using the GST Purification Kit (Cat. No. 635619).

GST is a 26-kDa whole protein that can be assayed biochemically as well as immunologically. This characteristic sets it apart from many epitope tags that are simply short peptides. However, GSTs large size results in a higher potential for degradation by proteases than other smaller tags. Therefore, performing GST-protein purification as quickly as possible under non-degrading conditions is necessary in order to minimize sample loss. 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 Compatibilities table in the Appendix when designing your purification scheme.

I. Introduction *continued*



The GST tag is specifically purified from the whole cell extract both as a fusion with DHFR and as a free protein. Whole cell extracts containing GST-DHFR (Panel A) and GST alone (Panel B) were loaded (arrow 1), washed with 1 X Extraction/Loading Buffer, and eluted (arrow 2) with Elution Buffer. Whole cell extract (lane 1), column flowthrough (lane 2, corresponding to peak I) and eluate (lane 3, corresponding to peak II) fractions were analyzed by SDS-PAGE (upper panels) and Western blot (lower panels) with an Anti-GST IgG.

II. List of Components

Store all components at 4°C.

The Glutathione-Superflow Resin is supplied as 50% (w/v) slurry in nonbuffered 20% ethanol.

Glutathione-Superflow Resin

Cat. No.	Size
635607	10 ml
635608	100 ml

Glutathione S-Transferase (GST) Purification Kit (Cat. No. 635619)

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

- **Five Glutathione-Superflow Columns**

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

  To prepare the 1X 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 10.23):

  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.**

III. Additional Materials Required

The following reagents are required but not supplied with the Glutathione-Superflow Resin:

- **Extraction buffer** (loading):
  140 mM NaCl; 10 mM Na_2HPO_4; 1.8 mM KH_2PO_4 (pH 7.5).
- **Elution buffer**:
  33 mM Glutathione in 50 mM Tris-HCl (pH 8.0). Prepare fresh.
- **Regeneration buffers**:
  First buffer: 0.1 M Tris-HCl; 0.5 M NaCl (pH 8.5).
  Second buffer: 0.1 M Sodium acetate; 0.5 M NaCl (pH 4.5).
  Third buffer: 140 mM NaCl; 10 mM Na_2HPO_4; 1.8 mM KH_2PO_4 (pH 7.5).
- **20% Ethanol Storage Solution**
  Dilute Ethanol to 20% in distilled water
- **Alumina** (Sigma Cat. No. 265497)[1]
- **Polypropylene tubes**
- **Centrifuge** (prechilled to 4°C)
- **TALON® 2-ml Disposable Gravity Column** (Cat. No. 635606)
- **Deionized H_2O**
- **Ice**
- **Column** (Pharmacia HR 10/2 or HR 10/10)[2]
- **Mortar/pestle**[1]
- **0.45-µm filter**[2]

[1]For alumina-based protein extractions.

[2]For FPLC applications

IV. Batch/Gravity-Flow Purification

PLEASE READ THE ENTIRE PROTOCOL BEFORE BEGINNING

A. Preparation of buffers

Prepare the buffers as specified in Additional Materials (Section III). If you have purchased the GST Purification Kit (Cat. No. 635619), dilute and dissolve the premade buffer solutions as specified in Section II.

B. Preparation of GST-fusion protein lysate

***NOTE**: Solutions containing GST should be kept at 4°C or on ice at all times.*

The method given below is generally applicable for up to 50 g of *E. coli* cell pellet containing GST-fusion. 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 Compatibilities table in the Appendix when designing your purification scheme.

1. Precool the mortar and pestle, centrifuge, and extraction buffer to 4°C. Prechill clean polypropylene tubes on ice to be used in Steps 4 & 5.
2. Transfer cell pellet containing your GST fusion protein to the precooled mortar.

   Note: Do not use more than 500 mg cell pellet per 1 ml Glutathione Resin.
3. Grind 1 part cells with 2.5 parts Alumina for 2–3 min, until the composition of the mixture is paste-like. (e.g., 500 mg cells: 1.25 g Alumina)
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, prechilled tube. **Do not disturb the pellet.** The supernatant is your clarified sample. If you have purchased the GST Purification Kit, proceed to Step D; otherwise, proceed to Step 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 20% Ethanol Storage Solution.

IV. Batch/Gravity-Flow Purification *continued*

2. Immediately transfer 2 ml of resin suspension to a TALON 2-ml Disposable Gravity Column (Cat. No. 635606) or any similar column. 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 upright, at 4°C or on ice to prechill the resin.

E. Batch/gravity-flow purification of GST protein

1. Add 1.5 ml of the clarified GST lysate (Step B.5) to the prechilled 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 prechilled extraction buffer to the column. **Do not disturb the resin**.
6. Repeat (Step 5) three times.
7. Elute your GST fusion protein by adding six 1 ml aliquots of elution buffer to the column. Collect the resulting eluate 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-electrophoresis to identify fractions containing your eluted GST fusion protein.

V. FPLC Purification

PLEASE READ THE ENTIRE PROTOCOL BEFORE BEGINNING

Before starting, prepare buffers as specified in Additional Materials (Section III). For the batch/gravity-flow purification protocol, see Section IV. Glutathione-Superflow Resin can be run at flow rates as high as 15 ml/cm^2/min for faster purification. However, slower flow rates of less than 1 cm/min are recommended for the *loading step* to allow maximal binding of the GST fusion.

A. Preparation of GST-fusion protein lysate

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

The method given below is a generally applicable, mild extraction method for up to 50 g of *E. coli* cell pellet containing a GST-fusion of interest.

***NOTE**: The extraction/loading buffer provided in the kit is sufficient for up to 2 g of E.coli pellet per purification. For larger amounts of E.coli pellet, you will need additional extraction/loading buffer not provided in the GST Purification Kit.*

Other extraction methods, such as sonication of cells on ice or use of lytic buffers can also be used, but may vary in recovery and yield.

1. Precool the mortar and pestle, centrifuge, and extraction buffer to 4°C. Prechill 2 clean polypropylene tubes on ice for use in Steps 4 & 5.
2. Transfer cells that express your GST fusion protein to the precooled mortar.
3. Grind 1 part cells with 2.5 parts Alumina (Sigma Cat. No. A-2039) for 2–3 min, until the composition of the mixture is paste-like (e.g., 500 mg cell pellet: 2.5 g Alumina).
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 prechilled tube. **Do not disturb the pellet.** The supernatant is your clarified sample. Proceed to Section B.

V. FPLC Purification *continued*

B. Preparation of Glutathione Resin for FPLC purification

1. We recommend a column whose internal diameter is at least 1 cm. Columns such as 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. For best results, use a column bed length of at least 3 cm. Use at least 1 ml of resin for every 100–500 mg of cell pellet (Step A.2).
2. Assemble and pack the column according to its manufacturer's specifications. We recommend a linear flow rate of at least 5 cm/min for packing.

   Note: 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 protein

1. In order to prevent clogging and extend the life of the column, filter the sample through a 0.45-µm filter before loading on the FPLC 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 unbound material in the flow-through 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. Ideally, to minimize chances for degradation of the sample, 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.

VI. Regeneration of Glutathione-Superflow Resin

Note: If you will not be using the column immediately after regeneration of the resin, complete steps 1–2, skip Step 3, and proceed directly to Step 4.

1. Wash the column/resin with approximately 10 resin volumes of the first regeneration buffer, followed by approximately 10 resin volumes of the second regeneration buffer.
2. Repeat the above wash cycles twice.
3. Equilibrate the column/resin with 10 resin volumes of the third regeneration buffer.
4. Store resin in 20% Ethanol Storage Solution as a slurry at 4°C.

VII. References

Bradford, M. (1976) A Rapid and Sensitive Method for the Quantitation of Microgram Quantities of Protein Utilizing the Principle of Protein-Dye Binding. *Anal. Biochem*. **72:**248–254.

Appendix: Reagent Compatibilities

Reagent Compatibilities	
Reagent	**Acceptable Concentration**
β-Mercaptoethanol	10 mM (with caution)
CHAPS	-
DTT, DTE	-
EDTA, EGTA	10 mM
Ethanol	30%, Regeneration Only
HEPES	50 mM
Glycerol	20%
Guanidinium	-
Imidazole	200 mM at pH 7.0–8.0
KCl	500 mM
MES	20 mM
MOPS	50 mM
NaCl	1.0 M
NP-40	1%
SDS	-
TRIS	50 mM
Urea	-

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This document has been reviewed and approved by the Clontech Quality Assurance Department.