G-Biosciences ♦ 1-800-628-7730 ♦ 1-314-991-6034 ♦ technical@GBiosciences.com

A Geno Technology, Inc. (USA) brand name

Glutathione Resin

(Cat. # 786-280, 786-310, 786-311, 786-312)

INTRODUCTION

The Glutathione Resin is designed for the single-step affinity purification of proteins with a glutathione *S*-transferase (GST) tag. The resin consists of reduced glutathione (GSH) coupled to 6% cross-linked agarose, via a 10 carbon spacer arm. This protocol can be adjusted conveniently to purify 100μg to 400mg of GST fusion protein.

ITEM(S) SUPPLIED

Cat. #	Description	Size
786-280	Glutathione Resin*	12.5ml
786-310	Glutathione Resin*	25ml
786-311	Glutathione Resin*	100ml
786-312	Glutathione Resin*	500ml

**Glutathione resin is supplied as 50% slurry in 20% ethanol*

STORAGE CONDITION

It is shipped at ambient temperature. Upon arrival, store refrigerated at 4°C, **DO NOT FREEZE**. This product is stable for 1 year at 4°C.

SPECIFICATIONS

- Binding Capacity: >40mg/ml resin
- Bead Structure: 6% cross-linked agarose
- Bead Size: 50-160μm

IMPORTANT INFORMATION

- *Sample preparation:* Refer to manufacturer's protocols for optimal conditions for growth, induction and lysis of recombinant GST-tagged clones.
- The purity and yield of the recombinant fusion protein is dependent of the protein's confirmation, solubility and expression levels. We recommend optimizing and performing small scale preparations to estimate expression and solubility levels.
- The amount of resin to use for a given crude lysate is dependent on the expression level of the GST protein and factors present in the lysate and lysis buffer that may affect binding. As a general guideline 50-200mg total protein lysate per ml of resin should be used.

ADDITIONAL ITEMS REQUIRED

Binding/Wash & Elution Buffers

- Binding/ Wash Buffer: 1X TBS or 1X PBS
- Elution Buffer: Binding / Wash Buffer with 10mM reduced glutathione (G-Biosciences Cat. # 786-588)

Regeneration Buffers

- RB1: 100mM Tris, 500mM NaCl, 0.1% SDS pH8.5
- RB2: 100mM sodium acetate, 500mM NaCl, 0.1% SDS, pH4.5

PREPARATION BEFORE USE

Sample preparation: Refer to manufacturer's protocols for optimal conditions for growth, induction and lysis of recombinant GST-tagged clones.

PROTOCOL

1. The table below is a guideline for the amount of resin and bacterial culture volumes that should be used. The actual amounts of GST fusion proteins obtained is highly variable and dependent of the fusion protein, host cell and culture conditions.

Protein yield	**100mg**	**10mg**	**1mg**	**100µg**
Culture Volume	25L	2.5L	250ml	25ml
Sonicate Volume	1.25L	125ml	12.5ml	1.25ml
Volume 50% Glutathione Resin	25ml	2.5ml	250µl	25µl
Elution Volume	12.5ml	1.25ml	125µl	12.5µl

2. The resin solution is supplied as 50% slurry and to make equilibrated 50% slurry, gentle shake the bottle to re-suspend the resin. Now, aliquot 1.33ml glutathione resin per 2ml 50% slurry required and sediment the gel by centrifugation at 500x *g* for 5 minutes.
3. Carefully decant the supernatant and then wash the resin with 10ml of cold 1X PBS per 1.33ml original glutathione resin.
4. Sediment the gel by centrifugation at 500x*g* for 5 minutes and carefully decant off the supernatant.
5. For each 1.33mls of original glutathione resin, add 1ml of 1 X PBS to make 50% slurry.
   NOTE*: Glutathione resin equilibrated in 1X PBS can be stored for 1 month at 4 ℃.*
6. Add 2ml of 50% slurry of glutathione resin to each 100ml of bacterial sonicate.
7. Incubate with gentle agitation at room temperature for 30 minutes.
   NOTE*: The resin can now be packed into disposable columns for easy washing and elution.*
8. Centrifuge at 500x*g* for 5 minutes and remove the supernatant.

9. Wash the resin with 5 volumes 1X PBS (1 volume is the starting amount of 50% glutathione resin).
10. Centrifuge at 500x*g* for 5 minutes and remove the supernatant. Repeat steps 9 and 10 twice.
    ***NOTE**: If desired, the fusion proteins may be cleaved while still bound to the gel with either thrombin or Factor Xa to free the protein of interest from the GST tag (see appendix). If not, proceed to next step for elution.*
11. Add 1ml elution buffer for each 2ml of starting 50% slurry.
12. Mix gently and incubate at room temperature to liberate the GST tagged protein.
13. Centrifuge at 500x*g* for 5 minutes and transfer the supernatant to a clean tube.
14. Repeat elution steps twice more, test the three fractions (analyzed by SDS-PAGE) and pool the enriched fractions.

RESIN REGENERATION

The resin/columns can be regenerated up to 5 times without loss of performance. To prevent cross-contamination use 1 column or resin for each specific protein being purified.

1. Wash resin with 10 bed volumes of RB1.
2. Wash resin with 10 bed volumes of distilled water.
3. Wash resin with 10 bed volumes of RB2.
4. Wash resin with 10 bed volumes of distilled water.
5. Store resin at 4°C in 20% ethanol.

APPENDIX: THROMBIN CLEAVAGE

***NOTE**: Samples should be removed throughout the digestion and analyzed by SDS-PAGE to estimate yield, purity and extent of digestion. The time, temperature and amount of thrombin required, varies for each GST tagged protein. Optimal conditions should be determined in a pilot experiment.*

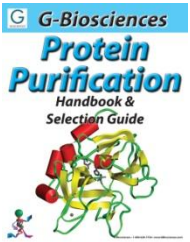
1. To elute the protein from the GST tag and agarose bead, add 10µl of thrombin (10 units) per mg GST tagged protein.
2. Mix gently and incubate at room temperature for 2-16 hours.
3. Once digestion is complete, GST can be removed by extensive dialysis against ~2000 volumes of 1X PBS, followed by purification on Glutathione Resin. The protein of interest will be in the flow-through.

TROUBLESHOOTING

Issue	Possible Cause	Suggested Solution
Low Protein Yield	Poor expression of soluble protein	Optimize bacterial expression and growth conditions. Check expression by SDS-PAGE to confirm expression.
	Protein insoluble and enters inclusion bodies	Try to limit inclusion body formation for inducing protein expression for shorter time periods or by performing inductions at 30°C. If inclusion bodies still form, follow the additional protocol for Inclusion Body Solubilization, using our Inclusion Body Solubilization (IBS) Buffer (Cat. # 786-183)
	The GST tag may not bind column	Supplement the lysis buffer with 5mM DTT before extraction may improve binding. Check the sequence of the construct to ensure the tag is in frame with the protein of interest. Test for presence of the His tag by Western blotting and probing with a α-His antibody
Protein Degradation	Protein is degraded by bacterial proteases	Use a protease inhibitor cocktail that does not use metal chelators. We recommend *Recom* ProteaseARREST™ (Cat. # 786-376), a protease inhibitor cocktail specific designed for purifying recombinant proteins from bacteria.
Poor Protein Purity	Poor column washing	Wash the column more than twice or try increasing the imidazole concentration.
	GST protein interacting with other proteins	Supplement the lysis buffer with 5mM DTT before extraction to help reduce non-specific interactions.
Slow Column Flow	Column overloaded or particulates added to column	Ensure the bacterial lysate is completely clear before adding resin, if necessary centrifuge the lysate a second time

RELATED PRODUCTS

Download our Protein Purification Handbook.



http://info.gbiosciences.com/complete-protein-purification-handbook/

For other related products, visit our website at www.GBiosciences.com or contact us.

Last saved: 3/20/2018 TN

www.GBiosciences.com