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A Geno Technology, Inc. (USA) brand name

Glutathione Resin Spin Columns

(Cat. # 786-714, 786-715, 786-716)

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 4% cross-linked agarose, via a 10 carbon spacer arm.

ITEM(S) SUPPLIED

Cat. #	Description	Total Column Volume	Size
786-714	Glutathione Resin, 0.2ml Spin Column	1ml	25 columns
786-715	Glutathione Resin, 1ml Spin Column	5ml	5 columns
786-716	Glutathione Resin, 3ml Spin Column	10ml	5 columns

**Glutathione Resin is supplied as a 50% slurry in 20% ethanol*

STORAGE CONDITIONS

It is shipped at ambient temperature. Upon arrival, store it 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: 4% 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 His-tagged clones. To avoid clogging of the resin filter the sample through a 0.45µm filter.

PROTOCOL

NOTE: *The total volume of the columns is indicated in the table above. For sample volumes larger than the column capacity, simply perform multiple applications and centrifugations until the entire sample has been applied. Ensure that the capacity of the column is not exceeded. The protocol below is for spin columns, however the columns can be used as gravity flow columns.*

1. Allow the columns to equilibrate to the appropriate purification temperature (4°C or room temperature).
2. Mix the protein sample with the Binding/Wash Buffer so the total volume is equivalent to two resin bed volumes (RBV). 0.4, 2 and 6ml for the 0.2, 1 and 3ml columns respectively.
3. Remove the bottom cap from the column and transfer to an appropriate centrifuge tube. 0.2, 1 and 3ml columns use 2, 15 and 50ml centrifuge tube respectively.
4. Centrifuge at 700xg for 2 minutes and discard the storage buffer.
5. Add two RBV Binding/Wash Buffer to the columns and allow to enter the resin bed.
6. Centrifuge at 700xg for 2 minutes and discard the Binding/Wash Buffer.
7. Add the protein sample to the columns and allow to enter the resin bed. For maximal binding incubate with mechanical end over end rotation for 30-60 minutes at room temperature or 4°C.
8. Centrifuge at 700xg for 2 minutes and collect the flow-through.
9. Wash the column with 2 RBV Binding/Wash Buffer.
10. Centrifuge at 700xg for 2 minutes and collect the wash. Repeat steps 9-10 two more times, collecting fractions in different tubes.
11. Elute the bound protein by the addition of 1 RBV of Elution Buffer.
12. Centrifuge at 700xg for 2 minutes and collect the elution. Repeat steps 11-12 two more times, collecting fractions in different tubes.

13. The elution of the protein can be monitored by measuring absorbance at 280nm or with a CB-X™ Protein Assay (Cat. # 786-12X). The fractions can also be analyzed by SDS-PAGE.
14. To remove the glutathione for downstream processing we recommend our SpinOUT™ GT-600 columns or our Tube-O-DIALYZER™ dialysis systems.

COLUMN REGENERATION

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

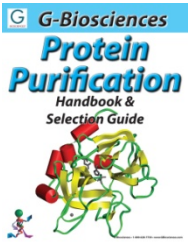
1. Wash resin with 10 bed volumes of RB1.
2. Wash resin with 10 bed volumes of distilled water.
3. Wash resin with 10 bed volumes of RB2.
4. Wash resin with 10 bed volumes of distilled water.
5. Store resin at 4°C in 20% ethanol.

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/

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