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A Geno Technology, Inc. (USA) brand name

Glutathione Resin Spin Plates

Immobilized Glutathione resin for
GST Tagged Protein Purification

(Cat. # 786-993)



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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.

The spin plate format are convenient, ready-to-use pre-dispensed filter plates for the rapid and efficient purification of GST tagged proteins from bacterial, mammalian and baculovirus infected cells. The plates are compatible with centrifugation and vacuum manifold systems for manual or automated purification. Ideal for fast, consistent well-to-well and plate-to-plate reproducibility for small scale high throughput purifications.

ITEM(S) SUPPLIED

Part. #	Description	Total Column Volume	Size
410G-A	Glutathione Resin Spin Plates	100µl resin/well	2 plates
111W-A	Wash/Collection Plates	-	4 plates

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

STORAGE CONDITIONS

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

- Plates are compatible with variable speed centrifuges with rotors and carriers capable of handling stacked plates. Use speed of 100-500xg with a maximum of 700xg.
- Ensure the spin plates are balanced throughout all centrifugations with a duplicate plate filled with an appropriate volume of water.
- The plates may be used with standard vacuum manifolds and is also dependent on sample properties and sample preparation. Use at a flow rate of 2-4psi (4-8 in Hg), which is equivalent to 1-2 props per second. To preserve the quality of the resin, avoid over-drying of the resin during vacuum application.
- 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.

- Each well has an estimated binding capacity of 1mg GST protein. The maximum total protein (lysate) loading amount is 4mg and typical yields are 10-25% of total protein loaded. Do not exceed the capacity of the resin.
- We recommend our Recom ProteaseArrest™ (Cat. # 786-376, 786-436) for inhibiting proteases during the purification of recombinant proteins.
- Avoid strong reducing agents, DTT or β -mercaptoethanol.
- For recombinant proteins that are sequestered to inclusion bodies we recommend our IBS™ Buffer (Cat. # 786-183)

ADDITIONAL ITEMS REQUIRED

- Variable speed centrifuge with rotor and carriers capable of handling stacked plates (4.5cm height) at 500xg or a vacuum manifold.
- Binding Buffer, see protocol for details
- Ultra pure water
- EDTA-free protease inhibitor cocktail, we recommend Recom ProteaseArrest™ (Cat. # 786-376, 786-436)

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
- Optional: Plate or orbital shaker
- Optional: Additional plates for collecting and storing fractions
- Optional: SpinOUT™ GT-600 Spin Plates (Cat. # 786-989, 786-990) for desalting and buffer exchange of samples
- Optional: Sealing tape for 96-well plates (Cat. # 786-422)

PREPARATION BEFORE USE

Sample preparation: Refer to manufacturer's protocols for optimal conditions for growth, induction and lysis of recombinant GST tagged clones. To avoid clogging of the resin filter the sample through a 0.45 μ m filter. T

PROTOCOL WITH CENTRIFUGATION

NOTE: The total volume of the wells is <400µl/well. 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.

1. Allow the columns to equilibrate to the appropriate purification temperature (4°C or room temperature).
2. Mix the protein sample 1:1 with the Binding Buffer so the total volume is <400µl.
3. Remove the bottom seal from the plate(s) and place on the top of a wash/collection plate. Remove the top seal.
4. Place the plate assembly in the centrifuge with a 96 well carrier and centrifuge at 500xg for 3 minutes to remove the storage buffer. Discard the storage buffer.
5. Add 400µl ultra pure water to each well. Repeat centrifugation and discard the water.
6. Add 400µl Binding Buffer to each well and allow to enter the resin bed. Centrifuge as before and discard the Binding Buffer. Repeat this step once.
7. Add <400µl protein sample from step 2 to the columns and allow to enter the resin bed. For maximal binding incubate with moderate agitation on a plate shaker or orbital shaker for 15-30 minutes at room temperature or 4°C.
8. Centrifuge the plate assembly at 500xg for 3 minutes. Make sure the applied sample is drained from all wells. Discard the flow through or use additional collection plates to store the flow through for further analysis.
9. Place the plate back on the wash/collection plate and wash the resin with 400µl/well of Wash Buffer.
10. Centrifuge at 500xg for 3 minutes and discard the wash. Repeat the wash three more times. Additional wash steps may be required for some samples.
11. Place the spin plate on a new collection plate. Add 200µl Elution Buffer and incubate with gentle agitation for 1 minute.
12. Centrifuge at 500xg for 3 minutes and collect the eluate. Repeat the elution step once for maximum concentration or twice for maximum yield. The elution volume can be varied to achieve maximum concentration. Use additional plates for collecting and storing fractions.
13. Seal collection plate top with sealing tape and store at 4°C.

14. 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.
15. To remove the glutathione for downstream processing we recommend our SpinOUT™ GT-600 Spin Plates (Cat. # 786-989, 786-990) for desalting and buffer exchange of samples.

PROTOCOL WITH VACUUM MANIFOLD

NOTE: *The total volume of the wells is <400µl/well. For sample volumes larger than the column capacity, simply perform multiple applications and vacuum applications until the entire sample has been applied. Ensure that the capacity of the column is not exceeded.*

1. Adjust the vacuum to obtain a flow rate of 4-8 inches Hg (2-4psi), the equivalent of 1-2 drops/second.
2. Allow the columns to equilibrate to the appropriate purification temperature (4°C or room temperature).
3. Mix the protein sample 1:1 with the Binding Buffer so the total volume is <400µl.
4. Remove the bottom seal from the plate(s) and then the top seal and place onto the top of the vacuum manifold. Apply vacuum to remove storage solution. Let the solution drain or collect in a wash/collection plate. Discard the storage buffer.
5. Add 400µl ultra pure water to each well. Apply vacuum and let drain or collect in the wash/collection plate and discard.
6. Add 400µl Binding Buffer to each well and allow to enter the resin bed. Apply vacuum and let drain or collect in the wash/collection plate and discard. Repeat this step once.
7. Add <400µl protein sample from step 2 to the columns and allow to enter the resin bed. For maximal binding incubate with moderate agitation on a plate shaker or orbital shaker for 15-30 minutes at room temperature or 4°C.
NOTE: *Detergent lysis solutions may foam and result in cross contamination. Lowering the flow rate can help alleviate this.*
8. Apply vacuum and let drain or collect in the wash/collection plate. Make sure the applied sample is drained from all wells. Discard the flow through or use additional collection plates to store the flow through for further analysis.
9. Place the plate back over the wash/collection plate on the vacuum manifold and wash the resin with 400µl/well of Wash Buffer.

10. Apply vacuum and let drain or collect in the wash/collection plate and discard. Repeat the wash three more times. Additional wash steps may be required for some samples.
11. Place the spin plate on a new collection plate. Add 200µl Elution Buffer and incubate with gentle agitation for 1 minute.
12. Place the plate and new wash/collection plate on the vacuum manifold. Apply vacuum and collect the eluate in the wash/collection plate. Repeat the elution step once for maximum concentration or twice for maximum yield. The elution volume can be varied to achieve maximum concentration. Use additional plates for collecting and storing fractions.
13. Seal collection plate top with sealing tape and store at 4°C.
14. 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.
15. To remove the glutathione for downstream processing we recommend our SpinOUT™ GT-600 Spin Plates (Cat. # 786-989, 786-990) for desalting and buffer exchange of samples.

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.

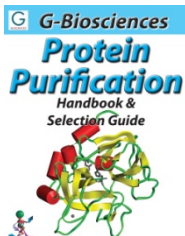
1. Wash resin with 10 bed volumes of RB1.
2. Wash resin with 10 bed volumes of distilled water.
3. Wash resin with 10 bed volumes of RB2.
4. Wash resin with 10 bed volumes of distilled water.
5. Store resin at 4°C in 20% ethanol.

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 <i>Recom</i> 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.

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