ABT

PROCEDURE FOR USE GLUTATHIONE AGAROSE BEADS Bulk Resins

DESCRIPTION

Glutathione Agarose Resin is used to purify recombinant derivatives of glutathione S-transferases or glutathione binding proteins.

Resins are products that allow batch or column purifications. Purification of GST fusion proteins using Glutathione Resin provides an easy one step purification.

This product is supplied as 75% (v/v) aqueous suspension (in 20% ethanol).

INSTRUCTIONS

I. Batch Purification of GST-tagged proteins

The following summarized procedure is adapted for the purification of GST-tagged protein in batch.

1. Elimination of the Preservative

Determine the quantity of Glutathione Agarose needed for your purification following the Recommendations below. Gently shake the bottle of Glutathione Agarose to achieve a homogeneous suspension. Immediately pipette the suspension (1.33 ml of the original Glutathione Agarose suspension per ml of gel volume required) to an appropriate tube. Sediment the gel by centrifugation at 500 x g for 5 minutes and carefully decant the supernatant and discard it.

Recommendations

Quantity of Glutathione Agarose required: Binding capacity will vary for each GST-tagged protein. The yield of GST-tagged protein depends on various parameters, such as nature of the fusion protein, expression host, culture conditions, etc.

Glutathione Agarose has an orientative binding capacity of 8 mg of GST tagged protein / ml gel(1).

(1) 1 ml gel corresponds to 1.333 ml of 75% (v/v) Glutathione Agarose suspension.

2. Equilibration of the Resin

Add 10 bed volumes of binding buffer to equilibrate the gel by mixing thoroughly to achieve a homogeneous suspension. Sediment the gel by centrifugation at 500 x g for 5 minutes and carefully decant the supernatant and discard it.

Note: Binding buffer: 10 mM Na_2HPO_4, 1.8 mM KH_2PO_4, 2.7 mM KCl, 140 mM NaCl, pH 7.3 (PBS buffer).

Note: A 50% suspension of pre-equilibrated Glutathione Agarose may be used directly or may be stored at 4ºC for up to 1 month and used if required.

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3. Application of the Sample

Once the resin is equilibrated, the sample containing the fused protein for purification is applied following the Recommendations below. Add the clarified *E. coli* lysate to the equilibrated resin and mix the suspension gently for 30 min at room temperature. In some cases a slight increase of contact time may facilitate binding.

Centrifuge the suspension at 500 x g for 5 minutes to sediment the resin. Carefully decant the supernatant and discard it.

Recommendations

Protein load and culture size

The expression level of GST-tagged proteins is high ranging from 10 [a] to 50 [b] mg/liter of *E. coli* culture.

Table 1: Required culture volumes for 1 ml of settled Glutathione Agarose [1].

Expression level	*E. coli* culture	Resuspend in	Protein lysate
10 mg / l [a].	800 ml culture (~ 3.2 g cell pellet [2])	~ 16 ml PBS [3]	~ 20 ml
50 mg / l [b]	160 ml culture (~ 0.64 g cell pellet [2])	~ 3.2 ml PBS [3]	~ 4 ml

(1) 1 ml of settled agarose corresponds to 1.333 ml of 75 % (v/v) Glutathione Agarose suspension.

(2). On average, 250 ml of culture will produce approximately 1g of pelleted, wet cells.

(3) 1g cells may be lysed in 2-5 ml PBS.

4. Washing of the Resin

Wash the gel by adding 10 ml bed volumes of binding buffer. Invert to mix and centrifuge the suspension at 500 x g for 5 minutes to sediment the resin. Carefully decant the supernatant and discard it.

Repeat the washing step twice (total wash 3 x 10 bed volumes of PBS).

Note: It will be washed with the binding buffer until the O.D. 280 nm is the same as the binding buffer.

5. Elution of the Pure protein

Add 1 bed volume of elution buffer to the gel. Mix thoroughly for 10 min at room temperature. Sediment the gel by centrifugation at 500 x g for 5 minutes and carefully decant or pipette the supernatant in a new tube and store on ice. Repeat the elution step twice or more and pool the fractions containing the purified protein.

Note: Elution buffer: 10 mM reduced glutathione in 50 mM Tris-HCl, pH 8.0. It is possible that a significant amount of GST fusion protein may remain bound to the resin. Conditions (volumes, times, temperatures) used for elution may vary among GST fusion proteins. Eluates should be monitored (Bradford protein assay, SDS-PAGE or measure the absorbance at 280 nm to determine the yield of the eluted GST-fused protein).

6. Regeneration & Storage

See the Procedure at the end of this publication.

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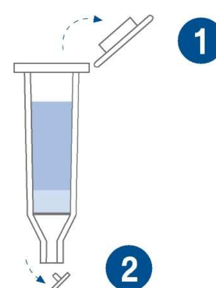
II. Gravity Purification of GST-tagged proteins

The following summarized procedure is adapted for the purification of GST-tagged protein in batch. We recommend to use our empty columns (Plastic Columns Cat. Nº. C-50 or Plastic XL Cat. Nº. CXL-50) depending on total volume size.

1. Elimination of the Preservative

Determine the quantity of Glutathione Agarose needed for your purification *(see section I.1-Recommendations).*

Gently shake the bottle of Glutathione agarose to achieve a homogeneous suspension. Immediately pipette sufficient suspension to an appropriate empty column [(1)].

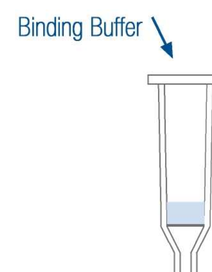


(1) Empty column information

Column	Cat. Nº	Total capacity
Plastic Columns	C-50	12 ml
Plastic Columns XL	CXL-50	35 ml

Remove first the upper and then the lower cap of the column, to allow elimination of the preservative by gravity flow.

2. Equilibration of the Pre-Packed Column



Equilibrate the column with 5ml bed volumes of binding buffer. Add the binding buffer on the upper part of the column and make sure no air has been trapped. Mix manually inverting the Pre-packed column and discard the supernatant. Repeat the equilibration step twice.

Note: Binding buffer: 10 mM Na_2HPO_4, 1.8 mM KH_2PO_4, 2.7 mM KCl, 140 mM NaCl, pH 7.3 (PBS buffer).

Note: A 50% suspension of pre-equilibrated Glutathione Agarose may be used directly or may be stored at 4ºC for up to 1 month and used if required.

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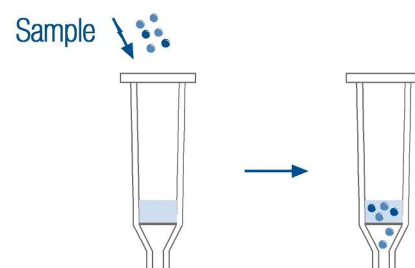
3. Application of the Sample



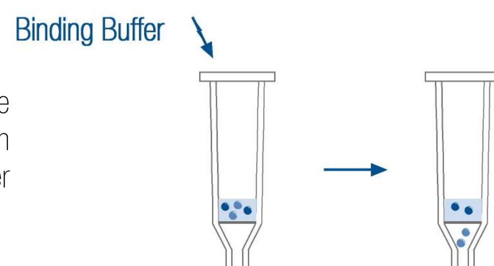
Add the sample containing the GST-tagged protein to be purified *(see section I.3-Recommendations)* through the top of the column, keeping sample and resin in contact at least 30 minutes before removing the bottom cap. Mix manually inverting the Pre-Packed column. Remove the lower cap of the column and discard the supernatant.

Note: In some cases a slight increase of contact time may facilitate binding.

Note: Pouring the sample down a glass rod held against the wall of the column will minimize the introduction of air bubbles.

Note: Binding capacity can be affected by several factors such as sample concentration.

4. Washing of the Pre-Packed Column

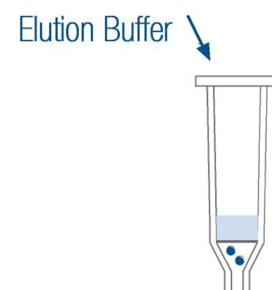


Close column outlet with the cap. Add the binding buffer (10 bed volumes) through the top to eliminate all the proteins that have not been retained in the column. Close column inlet with the cap, mix manually inverting the Pre-Packed column. Remove the lower cap of the column and discard the supernatant.

Repeat the washing step twice (total wash 3 x 10 bed volumes of PBS).

Note: It will be washed with the binding buffer until the O.D. 280 nm was the same as the binding buffer.

5. Elution of the Pure protein



Close column outlet with the cap. Add 1 bed volume of elution buffer to the column. Close column inlet with the cap and mix thoroughly for 10 min at room temperature. Sediment the gel, remove the end cap and collect the eluate in a new tube and store on ice.

Repeat the elution step twice and pool the collected eluates.

Note: Mix manually inverting the Pre-Packed column. It is advisable to keep elution buffer and resin in contact at least 10 minutes before removing the bottom cap.

Note: Elution buffer is 10 mM reduced glutathione in 50 mM Tris-HCl, pH 8.0.It Is possible that a significant amount of GST fusion protein may remain bound to the resin. Conditions (volumes, times, temperatures) used for elution may vary among GST fusion proteins. Eluates should be monitored (Bradford protein assay, SDS-PAGE or measure the absorbance at 280 nm to determine the yield of the eluted GST-fused protein).

6. Regeneration & Storage

See Procedure at the end of this Publication.

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III. Spin Purification of GST-tagged proteins

The following summarized procedure is adapted for the purification of GST-tagged protein in spin columns [(1)]. We recommend to use our empty Spin Columns (Cat. Nº. SP-25).

Note: 50 µl Gluthatione Agarose volume are used to purify up to 400 µg of GST-fused protein.

(1) In this protocol are required Empty mini spin columns with inserted frits of 10 - 20 µm pore size.

1. Elimination of the Preservative

Gently shake the bottle of Glutathione agarose to achieve a homogeneous suspension. Remove first the upper inlet cap and immediately pipette 67 µl of the original suspension to the empty spin column. Remove the lower outlet cap and put the spin column in a collecting tube. Centrifuge at 500 x g for 30 seconds.

Note: 67 µl of the original 75% suspension corresponds to 50 µl of gel.

2. Equilibration of the Spin Column

Equilibrate the spin column with 500 µl of binding buffer. Mix manually, centrifuge at 500 x g for 30 seconds and discard flow through.

Note: Binding buffer: 10 mM Na_2HPO_4, 1.8 mM KH_2PO_4, 2.7 mM KCl, 140 mM NaCl, pH 7.3 (PBS buffer).

Note: A 50% suspension of pre-equilibrated Glutathione Agarose may be used directly or may be stored at 4ºC for up to 1 month and used if required.

3. Application of the Sample

Close spin-column outlet with cap. Add the sample containing the GST-tagged protein to be purified (700 µl of clarified *E. coli* lysate) through the top of the spin column, keeping sample and resin in contact at least 30 minutes before removing the bottom cap. Mix manually inverting the spin column. Remove bottom cap and place spin column in a collecting tube.

Centrifuge at 500 x g for 30 seconds and discard the flow through.

Note: In some cases a slight increase of contact time may facilitate binding. Binding capacity can be affected by several factors such as sample concentration.

4. Washing of the Spin Column

Add 500 µl binding buffer through the top to eliminate all the proteins that have not been retained in the column. Mix manually inverting the Spin column.

Centrifuge at 500 x g for 30 seconds. Discard flow through. Repeat the washing step twice (total wash 3 x 500 ml of PBS). Discard flow through between washing steps.

Note: Wash the column with binding buffer until the O.D. 280 nm of the eluent reaches the baseline level.

5. Elution of the Pure protein

Close spin column outlet with cap. Add 50 µl of elution buffer and close the lid. Mix thoroughly for 10 min at room temperature. Centrifuge the gel, remove the end cap and collect the eluate in a new tube and store on ice. Repeat the elution step twice. Pool the collected eluates.

Note: Elution buffer is 10 mM reduced glutathione in 50 mM Tris-HCl, pH 8.0.It Is possible that a significant amount of GST fusion protein may remain bound to the resin. Conditions (volumes, times, temperatures) used for elution may vary among GST fusion proteins. Eluates should be monitored (Bradford protein assay, SDS-PAGE or measure the absorbance at 280 nm to determine the yield of the eluted GST-fused protein).

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6. Regeneration & Storage

See the Procedure at the end of this publication.

IV. FLPC Purification of GST-tagged proteins

Glutathione Agarose Resin is compatible with common low pressure chromatography columns and FPLC™ applications. We recommend columns equipped with an adjustable plunger/flow adapter. Use low rates for loading step to allow maximal binding of the GST-tagged protein. The flow rate for equilibration, washing and elution can be increased to reduce the purification time (see Table 1).

1. Column Packaging, Elimination of the preservative and Equilibration of the Resin

a. Manually shake the bottle to obtain a homogeneous suspension of Glutathione Agarose Beads/preservative. Place a funnel in the head of column and slowly run the suspension down the walls of the column.

   Note: It is advisable to make the addition slowly to avoid the formation of bubbles. The product may also be degassed before adding to the column.

   Decant the product and discard most of the leftover liquid, leaving 1 cm above the column head to prevent drying out. This is done either by passing it through the column, or pipetting it from the top of the column.

b. Repeat previous steps until the desired column height is obtained.

c. Insert the adapter gently in the column head until it begins to displace the liquid.

   Note: Make sure no air is trapped under the net.

d. Add distilled water to the purification stream until a constant height (corresponding to the height of the column) is achieved.

   Note: If the desired height is not achieved, repeat steps 1 through 4.

e. When a constant height has been obtained, maintain the flow with the addition of 5 volumes of distilled water to completely eliminate the preservative.

f. Equilibrate the column with at least 5 column volumes of binding buffer until the baseline at 280 nm is stable.

   Note: It is advisable to previously de-gas all the solutions before adding to the column to avoid the formation of bubbles.

Table 1: WORKING CONDITIONS

		Volumetric flow rate (ml / min)		
Column diameter (mm)	Bed volume (ml)	Packing	Equilibration/Washing/Elution	Binding
6.6	1	1.4	1	0.3 - 1
16	10	7	5	0.5 - 5
		Linear flow rate (cm/h) [1]		
		≤ 250	≤ 180	≤ 180

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(1) Converting from linear [cm/h] to volumetric flow rates [ml/min].

Converting from linear flow rate [cm/h] to volumetric flow rate [ml/min].

$$VF\,[ml/min] = \frac{LF\,[cm/h]}{60} \times A\,[cm^2] = \frac{LF\,[cm/h]}{60} \times \frac{\pi \times (d[cm])^2}{4}$$

(2) Converting from volumetric flow rate [ml/min] to linear flow rate [cm/h].

$$LF\,[cm/h] = \frac{VF\,[ml/min] \times 60}{A\,[cm^2]} = \frac{VF\,[ml/min] \times 60 \times 4}{\pi \times (d\,[cm])^2}$$

3. Application of the Sample

Once the resin is equilibrated, the centrifuged or filtered sample is applied *(see section I.3- Recommendations)*. In some cases a slight increase of contact time may facilitate binding. Therefore use low rates for the loading step to allow maximal binding of the GST-tagged protein.

Note: Binding capacity can be affected by several factors, such as sample concentration or the flow rate during sample application. Collect flow through and verify that GST has bound.

4. Washing of the Resin

It will be washed with the binding buffer (5 – 10 bed volumes of PBS) until the O.D. 280 nm reaches the baseline level.

5. Elution of the Pure protein

Elute the GST-tagged protein with 5-10 bed volumes of Elution buffer and collect the fractions on ice.

Note: Is important to identify (SDS-PAGE & Bradford protein assay) the fractions that contains the majority of pure protein.

6. Regeneration & Storage

See the Procedure below.

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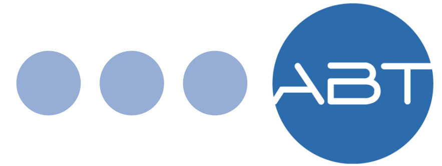

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REGENERATION PROCEDURE AND STORAGE

During the life of the resin, it may lose binding points because some protein is retained. Hence a loss of the binding capacity may be observed in successive cycles. To return to the starting state, regeneration may be necessary.

In general, column regeneration is always necessary when changing proteins. When continuing with the same protein it is recommended to do a regeneration when an appreciable diminution in the yield is observed. The frequency of these stages varies with the protein and the conditions used.

After use, regenerate the resin by sequentially applying 10 bed volumes of 100 mM Tris-HCl, 0.5 M NaCl, pH 8.5 followed by a second step with 10 bed volumes of 100 mM sodium acetate, 0.5 M NaCl pH 4.5. Repeat the above wash cycles twice and finally wash with 5 bed volumes of binding buffer. If you will not be using the resin immediately wash with additional bed volumes of 20% ethanol and store at 4ºC.

For laboratory use only. Not for use in diagnostic or therapeutic procedures.

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