INSTRUCTION MANUAL

Glutathione Agarose Resin

Agarose for Affinity Purification of GST-Tag Fusion Proteins and Glutathione binding proteins

(Cat. No. 42172)

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1. Glutathione Agarose Resin

1.1. General information

Glutathione Agarose Resin is optimized for the purification of Glutathion-S-Transferase (GST)-tagged fusion proteins and other glutathione binding proteins either by batch or column format. The agarose matrix is supplied as 75 % (v/v) suspension in 20 % (v/v) ethanol.

1.2. Storage conditions

Store at +2 °C to +8 °C (35 °F – 46 °F). Do not freeze.
If stored at the recommended temperature, the product will be suitable for use until: see label.

2. Batch purification of GST-tagged proteins

2.1. Elimination of the preservative

Determine the amount of Glutathione agarose according to table Tab. 1.

Expression level	*E. coli* culture	Resuspended in	Volume protein lysate
10 mg/L	800 ml (ca. 3.2 g cell pellet[1])	ca. 16 ml PBS[2]	ca. 20 ml
50 mg/L	160 ml (ca. 0.64 g cell pellet[1])	ca. 3-2 ml PBS[2]	ca. 4 ml

[1]: On average, 250 ml Bacterial culture will yield ca. 1 g pelleted, wet cells.
[2]: 1 g pellet may be lysed in 2 – 5 ml PBS.

Tab. 1: Required culture volume for 1 ml Glutathione agarose matrix bed volume (corresponds to 1.333 ml of 75 % (v/v) agarose suspension).

- Shake the bottle of Glutathione agarose suspension gently to get a homogeneous suspension.
- Immediately pipette the suspension to an appropriate tube.
- Sediment the gel by centrifuation (5 min at 500x g).
- Decant the supernatant carefully and discard it.

Note: The binding capacity will vary for each GST fusion protein. The protein yield depends on various parameters, e.g. the nature of the fusion protein, expression host, culture conditions, etc.

2.2. Equilibration of the agarose matrix

Bindung buffer:
The binding buffer (PBS) contains
10 mM Na_2HPO_4 (SERVA Cat. No. 30200), 1.8 mM KH_2PO_4 (SERVA Cat. No. 26870), 2.7 mM KCl (SERVA Cat. No. 26868), 140 mM NaCl (SERVA Cat. No. 30183), pH 7.3

- Add 10 bed volumes of binding buffer to agarose matrix.
- Mix gently to get a homogeneous suspension.
- Sediment the matrix by centrifugation (5 min at 500x g).
- Decant the supernatant carefully and discard it.
- A 50 % (v/v) suspension of the pre-equilibrated matrix may be used directly or stored at + 4 °C (39 °F) for up to 1 month.

2.3. Sample application

- Add the clarified *E. coli* lysate to the equilibrated resin.
- Mix the suspension gently at room temperature for 30 min.
- In some cases a slight increase of contact time may facilitate binding.
- Sediment the resin by centrifugation (5 min at 500x g).
- Decant the supernatant carefully and discard it.

2.4. Washing of the agarose matrix

- Add 10 bed volumes of binding buffer to agarose matrix.
- Mix gently to get a homogeneous suspension.
- Sediment the matrix by centrifugation (5 min at 500x g).
- Decant the supernatant carefully and discard it.
- Repeat the washing step 2 times (total wash 3x 10 bed volumes PBS) or until the absorption A_{280nm} is identical to the binding buffer.

2.5. Elution of the fusion protein

Elution buffer:
The elution buffer contains
10 mM reduced glutathione (SERVA Cat. No. 23150) in 50 mM Tris/HCl, pH 8.0

- Add 1 bed volume of elution buffer.
- Mix thoroughly for 10 min at room temperature.
- Sediment the matrix by centrifugation (5 min at 500x g).
- Carefully decant or pipette the supernatant in a new tube and store on ice.
- Repeat the elution step at least 2 times.

- Determine the protein content of each fraction by absorption A_{280nm}, Bradford assay or SDS PAGE.
- Pool the fractions which contain the fusion proteins.

2.6. Regeneration and storage of the agarose matrix

Regeneration buffer:
The regeneration is performed with the following buffers
Buffer 1: 100 mM Tris/HCl, 500 mM NaCl (SERVA Cat. No. 23150), pH 8.5
Buffer 2: 100 mM Natriumacetat (SERVA Cat. No. 21249), 500 mM NaCl (SERVA Cat. No. 23150), pH 4.5

- Regenerate the resin by applying 10 bed volumes of **Buffer 1** sequentially followed by a second step with 10 bed volumes of **Buffer 2**.
- Repeat the above wash cycles twice.
- Finally, wash with 5 bed volumes of binding buffer.
- If not used immediately, an additional wash with 5 bed volumes of 20 % (v/v) ethanol and storage at +4 °C (39 °F) can be performed.

3. Purification of GST fusion protein using gravity flow affinity columns

3.1. Elimination of the preservative

Determine the amount of Glutathione-Agarose according to table Tab. 1.

- Shake the bottle of Glutathione-Agarose suspension gently to get a homogeneous suspension.
- Immediately pipette the suspension to an appropriate empty column, e.g. Cat. No. 42174, 42175.
- Remove first the upper and the lower cap of the column, to allow elimination of the preservative by gravity flow.

Note: The binding capacity will vary for each GST fusion protein. The protein yield depends on various parameters, e.g. the nature of the fusion protein, expression host, culture conditions, etc.

3.2. Equilibration of the agarose matrix

Bindung buffer:
The binding buffer (PBS) contains
10 mM Na_2HPO_4 (SERVA Cat. No. 30200), 1.8 mM KH_2PO_4 (SERVA Cat. No. 26870), 2.7 mM KCl (SERVA Cat. No. 26868), 140 mM NaCl (SERVA Cat. No. 30183), pH 7.3

- Add 10 bed volumes of binding buffer to agarose matrix.
- Make sure that no air bubbles are trapped. To minimize the introduction of air bubbles use a glass rod.
- Mix manually by inverting the column.
- Remove the lower cap of the column, collect the flow through and discard it.
- A 50 % (v/v) suspension of the pre-equilibrated matrix may be used directly or stored at + 4 °C (39 °F) for up to 1 month.

3.3. Sample application

- Close the column outlet.
- Add the clarified *E. coli* lysate to the equilibrated resin.
- Close the column inlet.
- Mix the suspension gently at room temperature for 30 min.
- In some cases a slight increase of contact time may facilitate binding.
- Remove the lower cap of the column, collect the flow through and discard it.

3.4. Washing of the agarose matrix

- Close the column outlet.
- Add 10 bed volumes of binding buffer to agarose matrix.
- Close the column inlet.
- Mix gently to get a homogeneous suspension.
- Remove the lower cap of the column, collect the flow through and discard it.
- Repeat the washing step 2 times (total wash 3x 10 bed volumes PBS) or until the absorption A_{280nm} is identical to the binding buffer.

3.5. Elution of the fusion protein

Elution buffer:
The elution buffer contains
10 mM reduced glutathione (SERVA Cat. No. 23150) in 50 mM Tris/HCl, pH 8.0

- Close the column outlet
- Add 1 bed volume of elution buffer.
- Close the column inlet.
- Mix thoroughly for 10 min at room temperature.
- After sedimentation of the resin, remove the lower cap of the column, collect the flow through and store on ice.
- Repeat the elution step at least 2 times.
- Determine the protein content of each fraction by absorption A_{280nm}, Bradford assay or SDS PAGE.
- Pool the fractions which contain the fusion proteins.

3.6. Regeneration and storage of the agarose matrix

Regeneration buffer:
The regeneration is performed with the following buffers
Buffer 1: 100 mM Tris/HCl, 500 mM NaCl (SERVA Cat. No. 23150), pH 8.5
Buffer 2: 100 mM sodium acetate (SERVA Cat. No. 21249), 500 mM NaCl (SERVA Cat. No. 23150), pH 4.5

- Regenerate the resin by applying 10 bed volumes of **Buffer 1** sequentially followed by a second step with 10 bed volumes of **Buffer 2**.
- Repeat the above wash cycles twice.
- Finally, wash with 5 bed volumes of binding buffer.
- If not used immediately, an additional wash with 5 bed volumes of 20 % (v/v) ethanol and storage at +4 °C (39 °F) can be performed.

4. Purification of GST fusion protein using affinity spin columns

The following procedure is adapted for the purification of GST fusion proteins using spin columns. In this protocol empty mini spin columns with an inserted frit of 10 – 20 µm pore size, e. g. Cat. No. 42173, are required.
50 µl Glutathione agarose resin (corresponds to 67 µl suspension) are used to purify up to 400 µg of GST fusion protein.

4.1. Elimination of the preservative

- Shake the bottle of Glutathione agarose suspension gently to get a homogeneous suspension.
- Remove first the inlet cap and immediately pipette 67 µl of the suspension to an empty spin column.
- Remove the lower cap of the column and put the spin column in a collecting tube.
- Centrifuge at 500x g for 30 s.

Note: The binding capacity will vary for each GST fusion protein. The protein yield depends on various parameters, e.g. the nature of the fusion protein, expression host, culture conditions, etc.

4.2. Equilibration of the agarose matrix

Bindung buffer:
The binding buffer (PBS) contains
10 mM Na_2HPO_4 (SERVA Cat. No. 30200), 1.8 mM KH_2PO_4 (SERVA Cat. No. 26870), 2.7 mM KCl (SERVA Cat. No. 26868), 140 mM NaCl (SERVA Cat. No. 30183), pH 7.3

- Add 500 µl of binding buffer to the spin column.
- Mix manually by inverting the column.
- Remove the lower cap of the column and put the spin column in a collecting tube.
- Centrifuge at 500x g for 30 s.
- Collect the flow through and discard it.
- A 50 % (v/v) suspension of the pre-equilibrated matrix may be used directly or stored at + 4 °C (39 °F) for up to 1 month.

4.3. Sample application

- Close the column outlet.
- Add 700 µl the clarified *E. coli* lysate to the equilibrated resin.
- Close the column inlet.
- Keeping sample and resin in contact at least for 30 min at room temperature.
- In some cases a slight increase of contact time may facilitate binding.
- Remove the lower cap of the column and place spin in a collecting tube.
- Centrifuge at 500x g for 30 s.
- Collect the flow through and discard it.

4.4. Washing of the agarose matrix

- Close the column outlet.
- Add 500 µl of binding buffer to agarose matrix.
- Close the column inlet.
- Mix manually by inverting the column.
- Remove the lower cap of the column and put the spin column in a collecting tube, centrifuge at 500x g for 30 s.
- Collect the flow through and discard it.
- Repeat the washing step 2 times (total wash 3x 10 bed volumes PBS) or until the absorption A_{280nm} is identical to the binding buffer.

4.5. Elution of the fusion protein

Elution buffer:
The elution buffer contains
10 mM reduced glutathione (SERVA Cat. No. 23150) in 50 mM Tris/HCl, pH 8.0

- Close the column outlet.
- Add 50 µl of elution buffer.
- Close the column inlet.
- Mix thoroughly for 10 min at room temperature.
- Remove the lower cap of the column and put the spin column in a collecting tube, centrifuge at 500x g for 30 s.
- Collect the flow through and store on ice.
- Repeat the elution step at least 2 times.
- Determine the protein content of each fraction by absorption A_{280nm}, Bradford assay or SDS PAGE.
- Pool the fractions which contain the fusion proteins.

4.6. Regeneration and storage of the agarose matrix

Regeneration buffer:
The regeneration is performed with the following buffers
Buffer 1: 100 mM Tris/HCl, 500 mM NaCl (SERVA Cat. No. 23150), pH 8.5
Buffer 2: 100 mM sodium acetate (SERVA Cat. No. 21249), 500 mM NaCl (SERVA Cat. No. 23150), pH 4.5

- Regenerate the resin by applying 500 µl of **Buffer 1** sequentially followed by a second step with 500 µl of **Buffer 2**.
- Repeat the above wash cycles twice.
- Finally, wash with 250 µl of binding buffer.
- If not used immediately, an additional wash with 5 bed volumes of 20 % (v/v) ethanol and storage at +4 °C (39 °F) can be performed.

5. Trouble shooting

5.1. Sample application

Observation	Cause	Recommendation
Low protein yield	Problems with vector construction	Ensure that protein and tag are in frame.
	Poor protein expression	Optimize bacterial expression conditions
	Fusion protein forms inclusion bodies	Lower the growth temperature from 37 °C to 30 °C – 15 °C
	Insufficient extraction	Check extraction conditions (lysozyme, sonication) Use up to 2 % of a non-ionic detergent to improve cell disruption and/or protein solubilization
Highly diluted or concentrated sample	Highly diluted sample	Concentrate the sample before purification Perform adsorption step in batch format
	Highly concentrated sample	Dilute the sample before purification

5.2. Adsorption

Observation	Cause	Recommendation
Target protein does not bind efficiently	Sonication too severe	Choose milder sonication conditions. Over-sonication may cause conformational changes of the protein
	Missing reducing agent	Add Dithiothreitol (DTT, end concentration 5 mM) to the lysis buffer
	Highly diluted sample	Dilute the sample before purification
	Flow rate too high	Reduce flow rate. Perform adsorption step in batch format

Observation	Cause	Recommendation
Target protein does not bind efficiently	Channel formation in the resin	Re-pack the column
	Inadequate binding conditions	Check binding conditions
	Binding capacity exceeded	Apply less fused protein Regenerate resin Use fresh resin

5.3. Elution

Observation	Cause	Recommendation
Poor protein purity	Sonication too severe	Choose milder sonication conditions. Over-sonication may cause conformational changes of the protein
	Degradation of fusion protein	Perform purification at lower temperatures (+2 °C - +8 °C) Reduce purification step times Keep the samples and buffers on ice. Add protease inhibitors. Use protease-deficient host
	Insufficient washing step	Increase the number of washing steps with PBS
	Co-purification of chaperons	Add 5 mM $MgCl_2$ and 5 mM ATP to the lysate before purification
Target protein elutes poorly	Too smooth elution (low elution volume)	Increase volume of elution buffer
	Flow rate too high	Reduce flow rate. Perform purification in batch format
	Inadequate elution conditions	Check buffer and pH 50 mM reduced glutathione in the elution buffer improves elution

6. Ordering information

Columns					
Product	**Frit Pore Size**	**Resin Volume**	**Capacity**	**Cat. No.**	**Size**
Mini Columns	20 µm	100 - 250 µl	1.5 ml	42173.01 42173.02	25 columns 100 columns
Midi Columns	20 µm	0,5 – 2 ml	12 ml	42174.01	50 columns
Maxi Columns	20 µm	2 – 6 ml	35 ml	42175.01	50 columns
Mini Spin Columns	35 µm	50 - 100 µl	0.8 ml	42176.01	25 columns

Reagents		
Product	**Cat. No.**	**Size**
Na_2HPO_4	30200.01	500 g
KH_2PO_4	26870.01	500 g
KCl	26868.02	1 kg
NaCl	30183.01	1 kg
L-Glutathione red.	23150.02 23150.03 23150.04	5 g 25 g 100 g
Tris	37190.01 37190.02 37190.03	250 g 1 kg 5 kg
Natriumacetate	21249.02	500 g