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Glutathione MagBeads

Purification of GST (Glutathion-S-Transferase)-tagged Proteins

Product	Cat#	Package size
Glutathione MagBeads 5% suspension	S5392.0001	1mL
Glutathione MagBeads 5% suspension	S5392.0005	5mL
Glutathione MagBeads 5% suspension	S5392.0025	25mL

1. Overview

Genaxxon Glutathione MagBeads are developed for the affinity purification of glutathione-S-transferase (GST) fusion proteins. The affinity matrix is based on spherical magnetic agarose beads, consisting of 6% cross-linked agarose. The material is highly porous to allow optimal protein interaction. Cross-linked agarose is also physically very stable, making it suitable for purification processes without deformation or destruction. Our magnetic beads are very homogeneous in size with a medium particle diameter of 30 µm, yielding a high degree of reproducibility between individual purification runs. Glutathione is coupled to the magnetic agarose beads to obtain an affinity matrix with highest binding capacity for GST fusion proteins. Because the purification method depends on correctly folded GST protein, only native conditions can be used.

Magnetic beads are useful to purify proteins from dilute solutions, or when only low expression levels are achieved. Genaxxon Glutathione MagBeads are ferrimagnetic agarose beads coupled to glutathione, an efficient ligand for GST fusion proteins. A 5% suspension is provided. Genaxxon Glutathione MagBeads have a binding capacity of 8-10 mg protein/ml settled beads, and are produced in a very robust, reproducible way to minimize lot-to-lot variability.

Protein purification based on magnetic beads has become popular because they are useful to

- extract proteins from diluted solutions, such as cell culture supernatants
- highly reproducible results with low unspecific binding supernatants
- homogeneous in size and low lot-to-lot variation
- purify proteins expressed at low levels
- suited for purification from dilute samples and pull-down experiments

Genaxxon MagBeads enable fast and easy purification steps, which can be automated. The amount of magnetic beads used for a purification setup can be easily scaled up and down to match protein expression rates and culture volumes.

1.1 Specifications

particle size	30µm
pH stability	3.0-12.0
formulation	5% suspension in 20% ethanol
binding capacity*	8-10mg/mL settled beads
antimicrobial agent	20% ethanol
stability	2 years
storage	2°C - 8°C, do not freeze!

*as determined by purification of glutathione-S-transferase from *E.coli* cleared lysates, and quantified via spectrophotometry.

2. Purification of GST-tagged Proteins using Genaxxon Glutathione MagBeads

This protocol describes the generation of a cleared lysate from 200 ml of *E. coli* cell culture, and the purification of GST-tagged proteins under native conditions. The protocol may be modified depending on initial results which may have revealed (partial) insolubility of the GST-tagged protein or contamination of the preparation with unwanted proteins.
In this protocol, cell lysis is carried out using lysozyme, since it is inexpensive and very efficient for cells that had been frozen. However, lysis methods based on physical disruption (e.g., sonication or homogenization) or detergents (e.g., CHAPS) can also be applied. The GST-tagged target protein is purified under native conditions from the cleared *E. coli* lysate using Glutathione resin in a bind-wash-elute procedure. In this protocol binding is performed in batch mode using a magnetic separator, since it is the most efficient method, especially when the target protein is present only at low concentrations.

2.1

Equipment	Materials
☐ Centrifuge for 50 ml polypropylene tube, minimum 10.000 x g ☐ UV/VIS Spectrophotometer ☐ Micropipettors ☐ Micropipetting tips ☐ 15-ml polypropylene tubes, conical bottom (e.g. Falcon) ☐ 50-ml polypropylene tubes, conical bottom (e.g. Falcon) ☐ Magnetic holder for Microcentrifuge tube, for separation of magnetic beads ☐ pH meter ☐ End-over-end rotator	☐ Cell pellet from 200mL culture (typically 0.5g) ☐ Genaxxon Glutathione MagBeads (1mL; Genaxxon #S5392) ☐ Reduced glutathione (L-glutathione, reduced) ☐ ATP ☐ Magnesium sulphate ($MgSO_4$) ☐ Tris-hydrochloride (Tris-HCl, powder) ☐ Sodium chloride (NaCl) ☐ Benzonase® nuclease (e.g. Novagen®) ☐ Protease inhibitors ☐ Lysozyme ☐ EDTA ☐ Dithiothreitol (DTT) ☐ Sodium hydroxide (NaOH) ☐ Tris base ☐ HCl ☐ Triton X-100 ☐ Glycerol ☐ Sodium dodecyl sulfate (SDS) ☐ Bromophenol blue ☐ Urea (optional)

needed but not supplied

2.2 Solutions and Buffers

Lysis Buffer, 100mL

Component	Final concentration	Molecular weight (g/mol)	Stock concentration	Chemical needed for stock solution	Stock solution needed for buffer
TRIS base	125mM	121.14	5M	302.85g/500ml	2.5ml
NaCl	150mM	58.44	5M	146.1g/500ml	3ml
Protease inhibitors*	1x				2 tablets
Benzonase*	30U/ml buffer		25U/µl		120µl
Triton X-100	1% (v/v)		20%		5ml
DTT	1mM	154.25	1M	1.54g/10ml	100µl
EDTA	1mM	292.24	0.5M	1.46g/10ml	200µl
Lysozyme	1mg/ml		100mg/ml	1g/10 ml	1ml
Instructions: Dissolve in 90ml water. Adjust pH to 8.0 with HCl and add water to 100ml. Prepare fresh and use within 30 min of preparation. The use of protease inhibitors and Benzonase® is optional.					

Wash and Equilibration Buffer, 10mL

Component	Final concentration	Molecular weight (g/mol)	Stock concentration	Chemical needed for stock solution	Stock solution needed for buffer
TRIS base	125mM	121.14	5M	302.85g/500ml	250µL
NaCl	150mM	58.44	5M	146.1g/500ml	300µL
DTT	1mM	154.25	1M	1.54g/10ml	10µL
EDTA	1mM	292.24	0.5M	1.46g/10ml	20µL
Instructions: Dissolve in 8ml water. Adjust pH to 8.0 with HCl and add water to 10ml. Prepare fresh and use within 30 min of preparation.					

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ATP Buffer (optional), 10mL

Component	Final concentration	Molecular weight (g/mol)	Stock concentration	Chemical needed for stock solution	Stock solution needed for buffer
Tris-HCl, pH 7.4	50mM	121.14	1M	12.114g/100ml	0.5mL
ATP	2mM	551.14	100mM	5.51g/10ml	0.2mL
MgSO4	10mM	120.37	1M	12.37g/100ml	0.1mL
Instructions: Add water to 10 ml. Always prepare fresh.					

Elution Buffer, 10mL

Component	Final concentration	Molecular weight (g/mol)	Stock concentration	Chemical needed for stock solution	Stock solution needed for buffer
TRIS base	125mM	121.14	5M	302.85g/500ml	250µL
NaCl	150mM	58.44	5M	146.1g/500ml	300µL
Triton X-100	0.1% (v/v)		20%		50µL
Reduced glutathione	50 mM	307.32			weigh in 154mg
DTT	1mM	154.25	1M	1.54g/10ml	10µL
Instructions: Dissolve in 8ml water, stir until reduced glutathione is completely dissolved. Adjust pH to 8.0 with HCl and add water to 10ml. Prepare fresh and use within 30 min of preparation.					

5X SDS-PAGE Buffer, 10mL

Component	Final concentration	Molecular weight (g/mol)	Stock concentration	Chemical needed for stock solution	Stock solution needed for buffer
Tris-HCl, pH 6.8-7.0	300mM	121.14	1M	121.14g/1L	3mL
Glycerol	50% (v/v)	-	100% (v/v)	-	5mL
SDS	5% (w/v)	-	-	-	0.5g
Bromophenol blue	0.05% (w/v)	-	4%	-	125µL
DTT	250mM	154.25	1M	1.54g/10mL	125µL/aliquot
Instructions: Add water to 10ml, freeze 20 aliquots at -20 °C; before use, complete single aliquots with DTT at a final concentration of 250mM.					

2.3 Procedure

1. Thaw the pellet on ice for 15 min and resuspend a pellet derived from 200ml of *E. coli* culture in 1/20 Lysis buffer (in relation to culture volume).

2. Pour the resuspended pellet into a 50ml conical centrifuge tube and incubate on an end-over-end rotator at room temperature (15-25°C) for 30min. Optional: Add protease inhibitor to Lysis buffer. If the lysate is very viscious, add Benzonase® to Lysis buffer. Sonicate or French pressing the lysate to improve cell disruption is possible as well.

Optional: Freezing the resuspended cell pellet at -20°C for 30 min prior to 30 min. incubation at room temperature improves lysis by lysozyme.

3. Remove a 20µl sample, mix it with 5µl of 5× SDS-PAGE buffer, and store at −20°C for SDS-PAGE analysis. This total lysate sample can be used to analyze the expression of the GST-tagged protein.

4. Centrifuge the lysate at 10.000 × g at room temperature for 30 min.

5. Carefully collect the supernatant without touching the pellet. Remove a 20µl sample from the supernatant, mix it with 5µl of 5× SDS-PAGE sample buffer, and store at −20°C. This sample contains the soluble GST-tagged fusion protein and can be compared with the total lysate control to determine the solubility of the fusion protein.

Note: The supernatant contains the soluble proteins and is the **cleared lysate.**

6. Resuspend the Genaxxon Glutathione MagBeads by vortexing and transfer 40µl of the 25% magnetic bead suspension into a conical microcentrifuge tube. Place the tube on the magnetic

microtube stand until the beads are separated, and discard the supernatant.

7. Add 500µl of Equilibration and Wash buffer and mix by vortexing. Place the tube on a magnetic microtube stand until the beads are separated, and discard the supernatant. Repeat this step twice.

8. Add 1ml of the cleared lysate to the equilibrated resin and incubate at 4°C for 30 minutes, rotating end-over-end.

9. Place the tube on the magnetic microtube stand until the beads are separated and transfer the supernatant into a fresh conical microcentrifuge tube. Remove a 20µl sample from the flow-through fraction, mix it with 5µl of 5× SDS-PAGE sample buffer, and store at −20°C.

This is the **flow-through fraction.**

10. Remove the tube from the magnet, add 500µl of Wash buffer, mix by vortexing, incaubte for 30 seconds and put back on the magnetic microtube stand. Remove the supernatant. Remove a 20µl sample from the wash fraction, mix it with 5µl of 5× SDS-PAGE sample buffer, and store at −20°C. Repeat this step three times.

This is the **wash fraction.**

11. Elute the GST-tagged protein using 50 µl of Elution buffer and incubate for 5 minutes. Gently vortex the sample 2 or 3 times during incubation. Place the tube on the magnetic microtube stand until the beads are separated and transfer the supernatant into a fresh conical microcentrifuge tube. Remove a 4µl sample from the elution fraction, mix it with 1 µl of 5× SDS-PAGE sample buffer, and store at −20°C. Repeat this step 9 times and collect each eluate in a separate conical microcentrifuge tube.

This is the **elution fraction.**

12. Determine the protein concentration of each of the elution fractions with Bradford assay, using BSA as protein standard. Analyze all of the fractions by SDS-PAGE.

3. Tips and Tricks

- If cell disruption is inefficient using lysozyme only and sonification or mechanical disruption is not feasible, freezing the resuspended cell pellet at −20°C for at least 30 min prior to 30 min incubation at RT improves the cell lysis by lysozyme

- If the GST-tagged protein is not soluble under the recommended native buffer conditions and is mostly or completely in the insoluble fraction, or if it does not bind to the Glutathione Affinity matrix, you may try to destabilize conformation of the GST-tagged protein by adding the denaturant urea. Up to 4M urea has been successfully applied in the lysis and binding step and is, in principle, compatible with GST binding to immobilized glutathione. The concentration of urea or other reagents that is compatible with the target fusion protein may vary and must be determined empirically since the activity or structure of the protein might be affected by the inclusion of chaotropes or detergents.

-Cut the tip off a 1-ml pipette tip and pipet the resin slurry immediately to ensure transfer of the desired amount of resin bed volume.

-In some cases, a 65–70 kDa protein band from E. coli co-elutes with the target GST fusion protein from Glutathione Agarose. This well-known contaminant is most likely the chaperone DnaK. If an additional wash step is performed in the presence of ATP and MgSO4 (wash with 1 bv of ATP buffer), this protein is reliably and specifically removed, leaving the highly pure target fusion protein bound to the resin.

-Incubate the resin in elution buffer for 15 min before collecting the eluate; this can increase the protein concentration of the elution fraction. Apply 500 µl of elution buffer (1 bv) and immediately seal the column with the bottom outlet cap and incubate for 15min at room temperature (15-25°C); open the column and collect the eluate containing the GST-tagged protein.

- The number of elution steps required to completely elute the GST-tagged protein from the Glutathione Agarose can vary with the nature and amount of bound protein.

5. technical and performance parameters

pH stability	3-12	
chem. stability	HCl: 0.1M	SDS: 1% for 1h RT
	NaOH: 0.1M	2-Propanol
	Methanol: 100%	acetonitrile: 30% (v/v)
	Ethanol: 70%	HAc: 70%
	1M sodium acetate, pH4,0	
denaturating agents		Guanidinium hydrochloride: 6M
detergents	Triton X-100: 2%	Chaps: 1%
	Tween20: 2%	
additives	Imidazol: 2M	
	Ethanol: 20% + Glycerin: 50%	EDTA: <1mM; + MgCl2: <10mM
	Na_2SO_4: 100mM	citrate: 60mM
	NaCl: 1.5M	citrate: 60mM; + MgCl: 80mM
red. agents	red. glutathione: 1mM	

6. References

Schaefer F., Seip N., Maertens B., Block H., Kubicek J. Purification of GST-tagged proteins. (2011) Methods Navigator.

7. Important Information

Genaxxon Glutathione MagBeads are developed, designed and sold for research purposes only. It is not to be used for human, diagnostic or drug purposes or to be administered to humans unless expressly cleared for that purpose by the Food and Drug Administration in the USA or the appropriate regulatory authorities in the country of use. All due care and attention should be exercised in the handling of many of the materials described in this manual.

8. Warranty

Genaxxon guarantees only for the described properties of the Glutathione MagBeads over a period of 2 years (for Certificate of Analysis Date) if this product is used according to the information given in this publication. However, if you are not satisfied with this product, please contact Genaxxon Bioscience GmbH using given contact form or one of its authorized distributors.

9. Safety information

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate material safety data sheets (MSDSs). These are available online as pdf-file or on request (info@genaxxon.com).