Instructions for use

ROTI®Garose Glu/GST cartridges

Prefilled cartridges with agarose-Beads for direct one-step isolation of glutathione S-transferase(GST)-tagged molecules or other glutathione binding molecules by affinity chromatography.
For MPLC, FPLC, ÄKTA™ and peristaltic pump or syringe.

I. Characteristics

The bead matrix of ROTI®Garose-Glu/GST cartridges consists of beaded 4 % agarose without crosslinking, coupled with glutathione. Elution is carried out via a mild Tris/glutathione buffer, enabling even recovery of native protein complexes.
Efficient and highly specific isolation of GST-tagged proteins via affinity chromatography is based on the affinity of the glutathion-S-transferase to it's substrate glutathione. Merely no unspecific binding of proteins without GST-tag takes place, even during purification from raw extracts, while the biological activity of the purified proteins is preserved by chromatography conditions.
Recommended for isolation of glutathione-S-transferase (GST) or GST fusion proteins by FPLC.

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II. Suitablilty

	Glu/GST cartridges
Max. pressure	5 bar
Max. flow rate	5 ml/min
Recommended flow rate	0.5-5 ml/min
Ports	Standard (10-32)
Column material	PP and PE (frit)
Reducing conditions	not applicable
Bed volume	5 ml
Concentration	100 % beads
Preservative	20 % ethanol
Cross-linking	no
Agarose conc.	4 %
Ligand	glutathione
Binding capacity (approx..)	50 mg/cartridge *

* 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. The binding between GST-tag and glutathione is highly selective but slow. Thus, low flow rates and an increase in contact time during binding is strongly recommended in order to facilitate binding efficiency.

III. General notes

a) The following protocol has been optimised for the purification of GST-tagged molecules under native (mild, unreducing, undenaturing) conditions. However, these are general guidelines. Please optimise for each specific application.
b) Binding capacity of the resin is due to several factors, such as sample concentration, buffer composition etc. The binding between GST-tag and glutathione is highly selective but slow. Due to the optimised bead size, however, the flow through the matrix in general is fast.
Thus, low flow rates and an increase in contact time during binding is strongly recommended in order to facilitate binding efficiency.
c) In order to optimise the amount of protein isolated, we recommend to elute in bigger volumes and collect in fractions. Since the yield of eluted proteins decreases with time, fractions may be analysed separately regarding the yield, and pooled only if they contain significant amount of isolated protein.
d) In some cases, a significant amount of GST fusion protein remains 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.

IV. Purification of GST-tagged proteins by FPLC

Particular parameters are dependent on the molecules that are to be isolated, may vary from the protocol given here, and should be determined for each assay.

Connect cartridge to the chromatography system

1. Purge the pump with binding buffer (PBS, see VI. Buffers and general comments). Make sure that no air is trapped.
2. Remove the snap-off end at the column outlet and save it for further use. Remove the upper plug from the cartridge.
3. Fill the inlet port of the cartridge with several drops of buffer to remove air to form a positive meniscus. Start the pump and insert the fitting "drop-to-drop" into the cartridge port in order to avoid introducing air bubbles.
   Note: The snap-off end can be reused as a stop plug for a sealing the column outlet for storage.

Elimination of preservative and equilibration

4. Equilibrate the cartridge with 50-100 ml of PBS in order to completely eliminate the preservative.
   Note: Check the $OD_{280\ nm}$ of the flow through. Equilibrate until this OD reaches the baseline level of PBS.
   Note: A pre-equilibrated Glu/GST cartridge may be used directly or may be stored at 4 °C for up to 1 month and used if required.

Binding of GST-tagged proteins

5. Apply the clarified (filtred or centrifuged) *E. coli* lysate or protein extract to the column. Use *low* flow rates (0.5-2 ml/min) in order to allow maximal binding of the GST-tagged proteins.
   Note: Keep the flow through and check whether the protein has been successfully removed from the lysate. In case too much protein is still present,

reduce the flow rate further and apply the flow through again to the column.

6. Wash the column with 50 ml of PBS at a flow rate of 5 ml/min.
   Note: Check the $OD_{280\ nm}$ of the flow through. Wash until this OD reaches the baseline level of PBS.

Elution of target proteins

7. Elute the target proteins with 25-50 ml of elution buffer (see VI. Buffers and general comments) at a flow rate of 5 ml/min. Collect the flow through in fractions and keep on ice.
8. *Optionally:* Check the fractions for presence of the GST-tagged proteins (SDS-PAGE, Bradford) and pool the fractions containing the majority of the pure proteins.

V. Regeneration:

In order to preserve the optimum binding capacity during repeated binding and elution cycles, we recommend regenerating the resin once in a while. Regeneration frequency cannot be recommended in general, but should be performed if either the binding rate decreases, or when another protein is to be eluted.
Regeneration of the resin is performed by sequentially applying 50 ml of Regeneration buffer 1, followed by a second step with 50 ml of Regeneration buffer 2 ('regeneration washing cycle'). Repeat this regeneration washing cycle twice and finally wash with 25 ml of PBS.
In order to remove precipitated or denatured proteins, wash with 10 ml of Regeration buffer 3, followed by a washing step with 50 ml of PBS. If you will not be using the resin immediately wash with 25 ml of 20 % ethanol and store at 4 ºC.

VI. Buffers and general comments

Binding buffer: PBS buffer
We recommend to use ROTI®Stock 10x PBS buffer (Art. No. 1058). Or prepare: 10 mM NaH_2PO_4, 1.8 mM KH_2PO_4, 2.7 mM KCl, 140 mM NaCl, pH 7.3

Washing buffer: Same as binding buffer

Elution buffer: 10 mM reduced glutathione, 50 mM Tris-HCl, pH 8.0

Regeneration buffer 1: 100 mM Tris-HCl, 0.5 M NaCl, pH 8.5

Regeneration buffer 2: 100 mM sodium acetate, 0.5 M NaCl pH 4.5.

Regeneration buffer 3: 6 M guanidine hydrochloride

VII. Trouble Shooting

VII.A. Sample Application

Putative cause	Recommendation
Sample of high viscosity	
Presence of DNA in the sample	Sonify sample until viscosity is reduced or degrade DNA via DNAse
Steric hindrance of the substrate	Dilute the sample prior to application to the column. Purification in batch format may be method of choice.
Highly diluted or concentrated sample	
Highly diluted sample	Concentrate sample prior to application to the column. Adsorption in batch format may be the method of choice, followed by packing a blank column with the pre-adsorbed resin
Highly concentrated sample	Dilute sample prior to loading onto the column

VII.B. Adsorption

Putative cause	Recommendation
No binding of target protein to the column	
Inadequate binding conditions	Check buffers and binding pH. Check reagents used (best before dates). Use fresh chemicals. Recheck reagents used in binding buffer for interference with binding reaction. Check whether protein may be found in inclusion bodies.
GST-tag (or whole fusion protein) is not present or has been degraded	Purify protein at lower temperature (4°C). Reduce time needed for purification. Add Protease inhibitors. Double-check recombinant vector.
Extraction not optimal	Choose milder sonication conditions. Over-sonication can alter the conformation of the GST moiety.
	Add 5 mM DTT to the cell lysis buffer. Reducing agents may significantly increase the binding efficiency of some fusion proteins.
Resin used too often	Regenerate or use freh resin. Immobilized glutathione can be degraded by γ-glutamil-transpeptidase activity present in *E. coli* cell lysates.
Inefficient binding of target protein to the column	
Column capacity is exceeded	Apply less protein/sample. Increase matrix volume.
Matrix has been used too often	Apply a regeneration step
GST-tag is not binding well	Apply slower flow rates. Apply or increase incubation time of sample in the column. Choose batch format for binding in order to allow increased contact between resin and rec. protein. Chose buffer with denaturing conditions.
Protein concen-tration too low	Concentrate the sample prior to application
Flow rate too high	Use lower flow rate or skip to batch isolation in order to allow better contact between resin and fused protein
Matrix bed disturbed (channel-formation)	Use fresh column.

VII.C. Elution

Putative cause	Recommendation
High amount of co-eluted proteins (contaminants) / poor protein purity	
Insufficient washing	Increase volume of washing buffer
Inadequate adsorption conditions	Check above.
Co-purification of chaperonins	Several chaperonins involved in protein folding may co-purify with GST-fusion proteins, e.g. DnaK (~70 kDa), DnaJ (~37kDa), GrpE (~40 kDa), GroEL (~57 kDa), GrpE (~40 kDa), GroEL (~57 kDa), GroES (~10kDa). Co-purification of DnaK, for instance,

	can be avoided by treating the cell lysate with 5 mM $MgCl_2$ and 5 mM ATP prior to purification. For other chaperonins please see the common literature.
Target protein poorly eluted / low protein yield	
Too smooth elution conditions	Increase temperature moderately during elution. Reduce flow rate. Apply or increase binding time. Choose batch format for binding in order to allow increased contact between resin and fusion protein. Enhance volume of elution buffer and pool the fractions.
Flow rate too high	Decrease flow rate during elution
Inadequate elution conditions	Check formualtion and pH of the elution buffer. Use fresh reagents. For some fusion proteins, increase to up to 50 mM glutathione increases elution yield.
Problems with vector construction	Ensure that protein and tag are in frame
Poor protein expression	Optimize bacterial expression conditions. Check OD_{600} – overgrowth (too long incubation times) may dramatically lower protein yield.
Fusion protein forms inclusion bodies	Lower the growth temperature from 37 ºC to 30 ºC in maximum.
Extraction insufficient	Check extraction conditions (lysozyme, sonication). Use up to 2% of a non-ionic detergent to improve cell disruption and/or solubilization of the fusion protein.
Fusion proteins degraded	Sonication during extraction too severe. Chose milder conditions. Purify at lower temperatures (4 ºC). Reduce purification step times. Keep the samples and buffers on ice in order to reduce the activity of proteases. Add protease inhibitors. Use a protease-deficient host.
Elution profile is not reproducible	
Sample has been modified (e.g. lost tag)	Recheck conditions of bacterial growth / protein expression and sample preparation. Prepare fresh sample, standardise sample preparation. Run the protocol at 2-8 °C. Add protease Inhibitors.
Precipitation of proteins or lipids	Regenerate resin/column.
Buffers have changed	Prepare new buffers
Loss of binding capacity	Regenerate or use fresh resin. Immobilized glutathione can be degraded by γ-glutamil-transpeptidase activity present in *E. coli* cell lysates.

VIII. Storage
Store at +2 to +8 °C. Do not freeze.
Cartridges may not be autoclaved.

For research use only. Not approved for human or veterinary use, for application to humans or animals, or for use in clinical or in vitro *diagnostics.*

Warning H226-H319
P210-P280.7-P305+P351+P338

ROTI®Garose Glu/GST cartridges

1 x 5 ml	**0843.1**
5 x 5 ml	**0843.2**