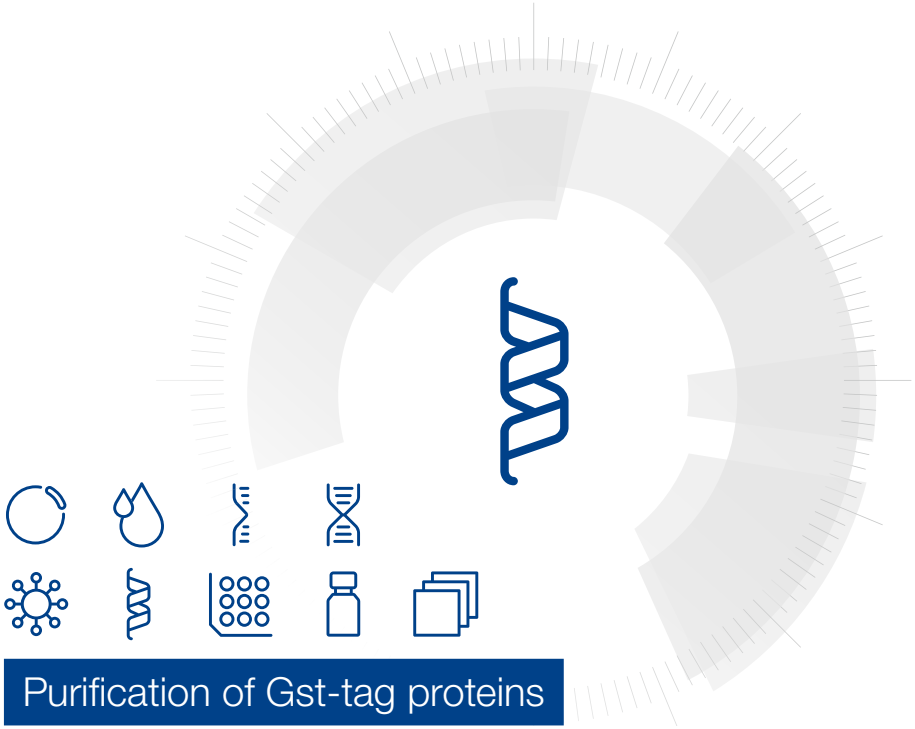
Bioanalysis

MACHEREY-NAGEL

User manual



Purification of Gst-tag proteins

■ Protino® Glutathione Agarose 4B

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1 Components

1.1 Kit contents and storage

	Protino® Glutathione Agarose 4B	
REF	**745500.10**	**745500.100**
Protino® Glutathione Agarose 4B	10 mL	100 mL
Volume of settled beaded agarose (bed volume)		
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Protino® Glutathione Agarose 4B is supplied as a 75 % (v/v) aqueous suspension containing 20 % ethanol to inhibit bacterial growth.

Shipping and storage of Protino® Glutathione Agarose 4B:

The product is shipped at ambient temperature.

Upon receipt Protino® Glutathione Agarose 4B should be **stored at 4 °C** and is stable up to 3 years. Do not freeze.

1.2 Additional material to be supplied by user

- PBS (10 mM Na_2HPO_4, 1.8 mM KH_2PO_4, 2.7 mM KCl, 140 mM NaCl, pH 7.3)
- Elution Buffer (50 mM Tris-HCl, 10 mM glutathione, pH 8)
- Lysozyme (required for cell extract preparation, see section 4.1)
- Appropriate centrifuge tubes, collecting tubes
- Appropriate centrifuge
- Spin columns or chromatography columns

1.3 About this user manual

It is strongly recommended for first time users to read the detailed protocol sections of the Protino®Glutathione Agarose 4B before using this product.

All technical literature is available online at ***www.mn-net.com***.

2 Product description

Protino® Glutathione Agarose 4B is an affinity chromatography medium which enables fast single step purification of glutathione-S-transferase (GST) fusion proteins and other glutathione-binding proteins. Purification of fusion proteins from a whole cell lysate is based on the strong affinity of the GST moiety for glutathione, which is immobilized on agarose beads. GST-tagged proteins are eluted under mild, non-denaturing, conditions using neutral-pH buffers containing free glutathione. The purification process preserves protein antigenicity and functionality. If removal of the GST-tag is necessary, fusion proteins may be cleaved using a site-specific protease.

Protino® Glutathione Agarose 4B can be used in batch binding studies and / or packed bed chromatography experiments. Packed columns may be operated by gravity flow or any liquid chromatography system such as FPLC™ system.

2.1 Specifications

Table 1: Specifications Protino® Glutathione Agarose 4B

Application	Batch binding Gravity flow column chromatography MPLC / FPLC™
Form	**75 %** (v/v) aqueous suspension containing 20 % ethanol 1 mL of settled agarose beads (1 mL bed volume) corresponds to 1.333 mL of original 75 % suspension
Matrix	4 % beaded agarose
Ligand	Glutathione, linked via sulfur atom
Spacer arm	12 atoms
Bead size	90 µm
Binding capacity[1]	> 8 mg recombinant GST/mL settled agarose
Recommended flow rates	*1 mL bed volume (column with 6.6 mm inner diameter)* Sample loading[2]: 0.2–1.0 mL/min Washing and elution: 1.0 mL/min *10 mL bed volume (column with 16 mm inner diameter)* Sample loading[2]: 0.5–5.0 mL/min Washing and elution: 5 mL/min
Maximum linear low rate[3]	250 cm/h
Chemical stability	Protino® Glutathione Agarose 4B withstands incubation in 0.1 M acetate pH 4, 0.1 M NaOH, 70 % ethanol, or 6 M guanidine hydrochloride for 2 hours at room temperature without significant loss of protein yield
Storage temperature	4–8 °C
Storage solution	20 % ethanol
Note	Do not autoclave the gel.

2.2 Culture size

The yield of GST-tagged proteins depends on various parameters, such as nature of the fusion protein, expression host, culture conditions, etc. However, some recommendations on protein load and culture size can be given (see Figure 1).

[1] Binding capacity will vary for each GST-tagged protein.
[2] Slow flow rates are recommended for the loading step to allow maximal binding of the GST-tagged protein.
[3] For converting from linear to volumetric flow rate see section 6.2.

Culture volume requirements are based on the following assumptions:

- Protino® Glutathione Agarose 4B has a binding capacity of 8 mg of recombinant GST per mL of settled agarose.
- Typically, the expression level of GST-tagged proteins is high ranging from 10 to 50 mg/L of E. coli culture.
- As a starting point we recommend to use the cell lysate from a 160–800 mL E. coli culture per 1 mL of settled Protino® Glutathione Agarose 4B.
- Since Protino® Glutathione Agarose 4B is supplied as 75 % (v/v) suspension, 1 mL of settled agarose beads (1 mL bed volume) corresponds to 1.333 mL of original 75 % suspension.

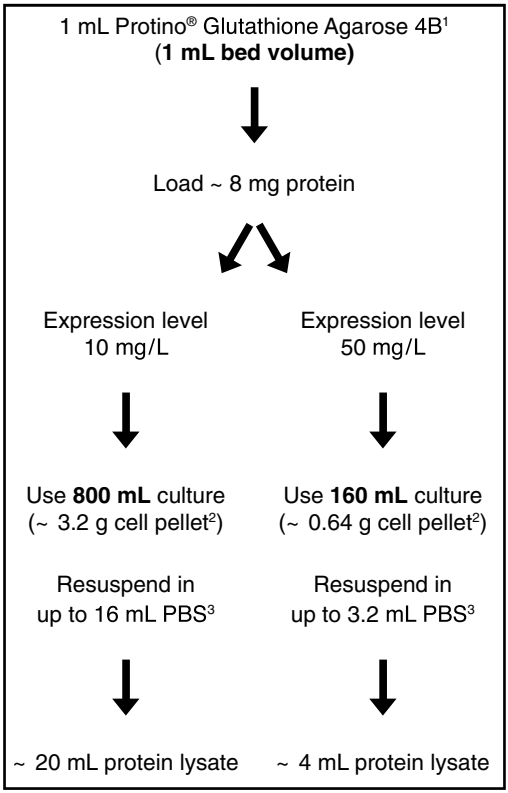

Figure 1 Required culture volumes for 1 mL settled Protino® Glutathione Agarose 4B1

[1] 1 mL bed volume corresponds to 1.333 mL of 75 % (v/v) Protino® Glutathione Agarose 4B suspension.
[2] On average, 250 mL of culture will produce approximately 1 g of pelleted, wet cells.
[3] 1 g cells may be lysed in 2–5 mL PBS, see section 4.1.

2.3 Preparation of buffers

Prepare the following working solutions.

For the preparation of cell lysates, **5 mL of PBS** per g of E. coli pellet wet mass are required.

Batch, gravity flow, spin column applications:

Per 1 mL of settled Protino® Glutathione Agarose 4B, approximately **50 mL of PBS** and **5 mL of Elution Buffer** are required for the purification procedure.

FPLC™ applications (1 mL bed volume):

10 mL of PBS are required to equilibrate the column, 10 mL of PBS are required to wash the column after sample application. 10 mL of Elution Buffer are required for the elution step.

*Note: that additional volumes of PBS and Elution Buffer must be prepared to flush lines and pumps depending on the chromatographic system: E.g., prepare approximately **250 mL of PBS** and **150 mL of Elution Buffer.***

Use high-purity chemicals and water for preparing the buffers. For best results, filter buffers through a 0.45 µm filter before use.

PBS (1 liter):

10 mM	Na_2HPO_4	1.780 g	$Na_2HPO4 \bullet 2\ H_2O$	M_r = 177.99 g/mol
1.8 mM	KH_2PO_4	0.245 g	KH_2PO4	M_r = 136.09 g/mol
2.7 mM	KCl	0.201 g	KCl	M_r = 74.55 g/mol
140 mM	NaCl	8.182 g	NaCl	M_r = 58.44 g/mol

Adjust pH to 7.3

Elution Buffer (1 liter):

50 mM	Tris base	6.06 g	Tris base	M_r = 121.14 g/mol
10 mM	glutathione	3.07 g	glutathione	M_r = 307.3 g/mol

Adjust pH to 8.0
Prepare fresh daily and store at 4 °C

3 Safety instructions

When working with the **Protino® Glutathione Agarose 4B** kit wear suitable protective clothing (e.g., lab coat, disposable gloves, and protective goggles). For more information consult the appropriate Material Safety Data Sheets (MSDS available online at ***www.mn-net.com/msds***).

The waste generated with the **Protino® Glutathione Agarose 4B** kit has not been tested for residual infectious material. Therefore, liquid waste must be considered infectious and should be handled and discarded according local safety regulations.

3.1 Disposal

Dispose hazardous, infectious or biologically contaminated materials in a safe and acceptable manner and in accordance with all local and regulatory requirements.

4 Protocols

4.1 Preparation of cleared *E. coli* lysates

1 Cultivate and harvest cells

- As a starting point we recommend to prepare 160–800 mL *E. coli* expression culture for the purification of 8 mg of GST-tagged protein using 1 mL bed of settled Protino® Glutathione Agarose 4B (see section 2.2).
- Harvest cells from an *E. coli* expression culture by centrifugation at 4,500–6,000 x *g* for 15 min at 4 °C. Remove supernatant.
- Cell pellets may be stored at -20 °C or -80 °C until needed.

2 Resuspend bacteria cells

- Thaw the cell pellet from an *E. coli* expression culture on ice (if frozen).
- Resuspend 1 g of pelleted, wet cells in 2–5 mL PBS. Pipette up and down, or stir until complete resuspension without visible cell aggregates. Perform this step on ice.

3 Lyse cells

- Add lysozyme to a final concentration of 1 mg/mL.
- Stir the solution on ice for 30 min.
- Sonicate the suspension on ice according to the instructions provided by the manufacturer (e.g., use 10 x 15 s bursts with a 15 s cooling period between each burst).
- Carefully check samples' appearance after sonication. If the lysate is still viscous from incomplete fragmentation of DNA, add 5 µg/mL DNase I and stir on ice for 15 min.

4 Clarify lysate

- Centrifuge the crude lysate at 10,000 x *g* for 30 min at 4 °C to remove cellular debris.
- Carefully transfer the supernatant to a clean tube without disturbing the pellet. If the supernatant is not clear, centrifuge a second time or filter through a 0.45 µm membrane (e.g., cellulose acetate).
- Store supernatant on ice.

Proceed to section

4.2 (Batch purification of GST-tagged proteins),

4.3 (Batch / gravity flow purification of GST-tagged proteins),

4.4 (Spin column purification of GST-tagged proteins), or

4.5 (FPLC™ purification of GST-tagged proteins).

4.2 Batch purification of GST-tagged proteins

Protino® Glutathione Agarose 4B is supplied as 75 % suspension. For batch purification of GST-tagged proteins, the original 75 % suspension can be used directly after performing the necessary equilibration steps (refer to step 1a). Alternatively, a 50 % preequilibrated working suspension can be produced (refer to step 1b) that may be used directly or may be stored at 4 °C for up to 1 month and used if required (refer to step 1c).

1a Equilibration (start with the 75 % original suspension)

- Determine the bed volume of Protino® Glutathione Agarose 4B required for your application (see section 2.2).
- Resuspend Protino® Glutathione Agarose 4B by mixing thoroughly to achieve a homogeneous suspension.
- Immediately transfer sufficient suspension to an appropriate tube. Pipette 1.333 mL of the original 75 % suspension per mL of bed volume required.
- Sediment the gel by centrifugation at 500 x *g* for 2–5 min. Carefully decant the supernatant (storage solution) and discard it.
- Add 10 bed volumes of PBS to equilibrate the gel. Invert to mix.
- Sediment the gel by centrifugation at 500 x *g* for 2–5 min. Carefully decant the supernatant and discard it.
- Proceed to step 2.

1b Preparation of a 50 % pre-equilibrated suspension (optional)

Protino® Glutathione Agarose 4B is supplied as 75 % suspension. It may be advantageous to prepare and store preequilibrated Protino® Glutathione Agarose 4B suspension, e.g. when several preparations are performed in parallel or when daily experiments are planned. The following steps produce a 50 % suspension of preequilibrated Protino® Glutathione Agarose 4B which may be used directly or may be stored at 4 °C for up to 1 month and used if required (refer to section 1c).

- Determine the bed volume of Protino® Glutathione Agarose 4B required for your application (see section 2.2).
- Resuspend Protino® Glutathione Agarose 4B by mixing thoroughly to achieve a homogeneous suspension.
- Immediately transfer sufficient suspension to an appropriate tube. Pipette 1.333 mL of the original 75 % suspension per mL of bed volume required.
- Sediment the gel by centrifugation at 500 x *g* for 2–5 min. Carefully decant the supernatant (storage solution) and discard it.
- Add 10 bed volumes of PBS to equilibrate the gel. Invert to mix.
- Sediment the gel by centrifugation at 500 x *g* for 2–5 min. Carefully decant the supernatant and discard it.

- Add 1 bed volume of PBS. Invert to mix. This results in a 50 % suspension of Protino® Glutathione Agarose 4B, which may be used directly or may be stored at 4 °C for up to 1 month.
- If the suspension is used directly proceed to step 1c.

1c Start with 50 % preequilibrated suspension

- Prepare 50 % preequilibrated suspension according to section 1b.
- Determine the bed volume of Protino® Glutathione Agarose 4B required for your application (see section 2.2).
- Resuspend Protino® Glutathione Agarose 4B by mixing thoroughly to achieve a homogeneous suspension.
- Immediately transfer sufficient suspension to an appropriate tube. Pipette 2 mL of the 50 % preequilibrated suspension per mL of bed volume required.
- Proceed to step 2.

2 Batch binding

- Add the clarified *E. coli* lysate to the equilibrated gel.
- Mix the suspension gently for 30 min at room temperature.
- Sediment the gel by centrifugation at 500 x *g* for 2–5 min. Carefully decant the supernatant and discard it.

3 Washing

Wash the gel by adding 10 bed volumes of PBS. Invert to mix.

Sediment the gel by centrifugation at 500 x *g* for 2–5 min. Carefully decant the supernatant and discard it.

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

4 Elution

Add 1 bed volume of Elution Buffer to the sedimented gel.

Mix the suspension gently for 10 min at room temperature to liberate the GST-tagged protein from the gel.

Sediment the gel by centrifugation at 500 x *g* for 2–5 min. Carefully decant or pipette the supernatant in a new tube and store on ice.

Repeat the elution step twice. Pool the collected eluates.

Note: The amount of elution buffer required may vary among fusion proteins. Volumes may have to be increased or additional elution steps may be necessary. Use a Bradford protein assay, SDS-PAGE or measure the absorbance at 280 nm to determine the yield of the eluted GST-tagged protein.

4.3 Batch / gravity flow purification of GST-tagged proteins

1 Equilibration

- Determine the bed volume of Protino® Glutathione Agarose 4B required for your application (see section 2.2).
- Resuspend Protino® Glutathione Agarose 4B by mixing thoroughly to achieve a homogeneous suspension.
- Immediately transfer the determined volume of suspension to an appropriate chromatography column (e.g., Protino® Columns 14 mL, 35 mL; see ordering information). Pipette 1.333 mL of the original 75 % suspension per mL of bed volume required.
- Allow the column to drain by gravity.
- To equilibrate the gel, add 10 bed volumes of PBS. Allow the column to drain by gravity. Avoid disturbing the resin.

2 Batch binding

- Close column outlet with cap or Parafilm®.
- Add clarified E. coli lysate to the gel and close column inlet with cap or Parafilm®.
- Mix the suspension gently for 30 min at room temperature.
- Remove bottom and top cap and allow the column to drain by gravity.

3 Washing

- To wash the gel, add 10 bed volumes of PBS. Allow the column to drain by gravity. Avoid disturbing the resin.
- Repeat the washing step twice (total wash 3x 10 bed volumes of PBS).

4 Elution

- Close column outlet with cap and add 1 bed volume of Elution Buffer.
- Close column inlet and mix the suspension gently for 10 min at room temperature to liberate the GST-tagged protein from the gel.
- Remove bottom cap and collect the eluate.
- Repeat the elution step twice. Pool the collected eluates.

Note: The amount of elution buffer required may vary among fusion proteins. Volumes may have to be increased or additional elution steps may be necessary. Use a Bradford protein assay, SDS-PAGE or measure the absorbance at 280 nm to determine the yield of the eluted GST-tagged protein.

4.4 Spin column purification of GST-tagged proteins

In this exemplary protocol mini spin columns (Receiver Columns 35 µm, see ordering information, section 6.3) with 50 µL Protino® Glutathione Agarose 4B bed volume are used to purify up to 400 µg of GST-tagged protein.

1 Equilibration

- Resuspend Protino® Glutathione Agarose 4B by mixing thoroughly to achieve a homogeneous suspension.
- Immediately transfer 67 µL of the original 75 % suspension to a Receiver Column placed in a collecting tube (67 µL of the original 75 % suspension corresponds to 50 µL of bed volume).
- Centrifuge at 500 x *g* for 30 s.
- To equilibrate the gel add 500 µL of PBS.
- Centrifuge at 500 x *g* for 30 s. Discard flowthrough.

2 Batch binding

- Close column outlet with cap.
- Add up to 700 µL of clarified E. coli lysate to the gel and close the lid.
- Mix the suspension gently for 30 min at room temperature, e.g., using an eppendorf Thermomixer.
- Remove bottom cap and place Receiver Column in a collecting tube.
- Centrifuge at 500 x *g* for 30 s. Discard flowthrough.

3 Washing

- To wash the gel, add 500 µL of PBS.
- Centrifuge at 500 x *g* for 30 s. Discard flowthrough.
- Repeat the washing step twice (total wash 3 x 500 µL of PBS). Discard flowthrough between washing steps.

4 Elution

- Close column outlet with cap. Add 50 µL of Elution Buffer and close the lid.
- Mix the suspension gently for 10 min at room temperature to liberate the GST-tagged protein from the gel.
- Remove bottom cap and place Receiver Column in a 1.5 or 2 mL micro-centrifuge tube.
- Centrifuge at 500 x *g* for 30 s.
- Repeat the elution step twice. Pool the collected eluates.

Note: The amount of elution buffer required may vary among fusion proteins. Volumes may have to be increased or additional elution steps may be necessary. Use a Bradford protein assay, SDS-PAGE or measure the absorbance at 280 nm to determine the yield of the eluted GST-tagged protein.

4.5 FPLC™ purification of GST-tagged proteins

Protino® Glutathione Agarose 4B, with a maximum flow rate of approximately 250 cm/h, is compatible with common low pressure chromatography columns and FPLC™ applications. We recommend columns equipped with an adjustable plunger / flow adapter. Binding kinetics between GST and immobilized glutathione is relatively slow. Therefore use low rates for the 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 2).

1 Preparing the chromatography system

- Purge the pump with PBS. Assure that all air is displaced.
- Determine the bed volume of Protino® Glutathione Agarose 4B required for your application (see section 2.2). Choose a appropriate chromatography column (e.g., from Omnifit or GE Healthcare). If more than 75 % of the column volume is to be packed, equip the column with an extension to hold the complete volume of the agarose suspension.
- Eliminate air from outlet tubing and end piece of the column by injecting PBS into outlet tubing. Close outlet of column. Leave ~ 1 cm of buffer above the support net or frit.
- Inject PBS into the inlet tubing of the upper plunger to eliminate air. Place plunger into a beaker containing PBS until use.

2 Column packing

- Resuspend Protino® Glutathione Agarose 4B by mixing thoroughly to achieve a homogeneous suspension. Immediately transfer the determined volume of suspension to an appropriate vacuum flask. Pipette 1.333 mL of the original 75 % suspension per mL of bed volume required and de-gas.
- Pour the entire slurry into the column in one continuous motion along a glass rod held against the inner wall of the column.
- Carefully fill the remaining space with PBS. Insert the upper plunger into the column without introducing air bubbles. Connect the inlet of the column to a pump.
- Open the column outlet and start the pump. Pass PBS through the column at a packing flow rate of approximately 250 cm/h (see Table 2 below) until height of gel bed becomes constant. Stop the pump and close the column outlet.
- Position the upper plunger on top of the column bed. Avoid to introduce air bubbles. Open the column outlet and start the pump at a flow rate of approximately 250 cm/h until the bed is stable. Re-position the plunger on the medium surface as necessary.

3 Column equilibration

- Equilibrate the column with approximately 5–10 bed volumes of PBS until the baseline at 280 nm is stable.

4 Binding

- Load the centrifuged or filtered sample onto the column.

NOTE: Binding kinetics between GST and immobilized glutathione is relatively slow. Therefore use low rates for the loading step to allow maximal binding of the GST-tagged protein.

- Collect flow through and analyze, e.g., by SDS-PAGE to verify that the GST-tagged protein has bound. If the fusion protein is found in early fractions of the flowthrough, the flow rate should be decreased. If the fusion protein is absent in early fractions and does appear in late fractions of the flow- through the column capacity has been exceeded. In this case protein load should be reduced or bed volume should be increased.

5 Washing

- Wash the column with 10 bed volumes of PBS or until the baseline at 280 nm is stable.

6 Elution

- Elute the GST-tagged protein with 10 bed volumes of Elution Buffer and collect fractions.
- Use a Bradford protein assay, SDS-PAGE, or measure the absorbance at 280 nm to identify the fraction(s) which contain(s) the majority of the eluted GST-tagged protein and analyze by SDS-PAGE.

Table 2: Recommended flow rates for Protino® Glutathione Agarose 4B

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

[1] For converting from linear to volumetric flow rate see section 6.2.

5 Regeneration and storage

Reuse of Protino® Glutathione Agarose 4B should only be performed with identical GST-tagged proteins to avoid possible cross-contamination. The lifetime of the matrix depends on the nature of the sample.

If a single GST-tagged protein is to be purified several times, simply wash with 10 bed volumes of PBS.

Basic cleaning: Wash resin with approximately 10 bed volumes of 100 mM Tris-HCl + 0.5 M NaCl, pH 8.5, followed by approximately 10 bed volumes of 100 mM sodium acetate + 0.5 M NaCl, pH 4.5. Repeat the above wash cycles twice. Wash with 5 bed volumes of PBS.

Rigorous cleaning: To remove precipitated or denatured proteins, wash with 2 bed volumes of 6 M guanidine hydrochloride, immediately followed by 5–10 bed volumes of PBS. To remove hydrophobically bound contaminants, wash with 4 bed volumes of 70 % ethanol or 1 % Triton X-100 followed by 5–10 bed volumes of PBS.

If you will not be using the matrix immediately, wash with additional 5 bed volumes of 20 % ethanol and store at 4 °C.

6 Appendix

6.1 Troubleshooting

Problem	Possible cause and suggestions
Low protein yield	*Problems with vector construction* • Ensure that protein and tag are in frame. *Low protein expression* • Optimize bacterial expression conditions. *Fusion protein forms insoluble aggregates (inclusion bodies)* • Lower the growth temperature from 37 °C to 30–15 °C. *Extraction may be 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 protein does not bind efficiently	*Sonication may have been to severe* • Choose milder sonication conditions. Over-sonication can alter the conformation of the GST moiety and prevents the fusion protein from binding to Protino® Glutathione Agarose 4B. *Reducing agent missing* • Adding DTT to the lysis buffer (final concentration 5 mM) prior to cell lysis can significantly increase binding of some fusion proteins. *Flow rate too high* • Decrease flow rate for the loading step to allow maximal binding of the GST-tagged protein. *Concentration of fusion protein is too dilute* • Concentrate the sample. Yield depends on the concentration of the fusion protein in the lysate. If the sample is too dilute, fusion proteins may not bind efficiently.
Fusion protein does not bind efficiently *(continued)*	*Protino® Glutathione Agarose 4B has been used several times* • Clean matrix according to section 5 or use fresh matrix. Immobilized glutathione can be degraded by -glutamyl transpeptidase activity in *E. coli* cell lysates. Therefore, matrices with immobilized glutathione have a finite lifetime.

Problem	Possible cause and suggestions
Fusion protein elutes inefficiently	*Low elution volume* • Increase the volume of Elution Buffer. Depending on the nature of the fusion protein and the amount of protein loaded, additional elution steps or buffer volume is required. *Flow rate too high* • Decrease flow rate during elution. *Incorrect buffer composition* • Check composition and pH of the Elution Buffer. In some cases up to 50 mM reduced glutathione may be used to improve elution. *Elution Buffer not prepared immediately before use* • Prepare Elution Buffer immediately before use.
Poor protein purity	*Insufficient washing* • Increase the number of washes with PBS. *Degradation of GST fusion protein* • Add a protease inhibitor to the lysis solution. Multiple bands may be the result of partial degradation by host proteases during the purification procedure. • Keep all samples and buffers on ice to reduce the activity of proteases. • Use a protease-deficient host. Multiple bands may be the result of partial degradation by host proteases during cell growth. *Sonication may have been too severe* • Choose milder sonication conditions. Over-sonication can lead to the co-purification of host proteins with the GST-tagged protein.
Poor protein purity *(continued)*	*Co-purification of chaperonins* • Several chaperonins, that are involved in protein folding, may co-purify with GST fusion proteins, for example, DnaK (~ 70 kDa), DnaJ (~ 37 kDa), GrpE (~ 40 kDa), GroEL (~ 57 kDa), GrpE (~ 40 kDa), GroEL (57 kDa), GroES (~ 10 kDa). Several additional purification steps have been described. For example co-purification of DnaK can be avoided by treating the cell lysate with 5 mM $MgCl_2$ and 5 mM ATP prior to purification. DnaK can be dissociated from other components in the presence of ATP and Mg^{2+}.

6.2 Converting from linear to volumetric flow rates and vice versa

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

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

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

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

LF	Linear flow rate [cm/h]
VF	Volumetric flow rate [mL/min]
A	Column cross-sectional area [cm^2]
d	Column inner diameter [cm]

6.3 Ordering information

Product	REF	Pack of
Protino® Glutathione Agarose 4B	745500.10	10 mL (settled agarose beads)
	745500.100	100 mL (settled agarose beads)
Protino® GST/4B Columns 1 mL	745510.5	5 columns
Protino® GST/4B Columns 5 mL	745515.1 745515.5	1 column 5 columns
Receiver Columns 35 µm	740524.10 740524.50 740524.250	10 columns 50 columns 250 columns
Protino® Columns 14 mL (without caps)	745250. 10	10 columns
Protino® Columns 35 mL (without caps)	745255. 10	10 columns

Visit ***www.mn-net.com*** for more detailed product information.

6.4 Product use restriction / warranty

Protino® Glutathione Agarose 4B products are intended, developed, designed, and sold FOR RESEARCH PURPOSES ONLY, except, however, any other function of the product being expressly described in original MACHEREY-NAGEL product leaflets.

MACHEREY-NAGEL products are intended for GENERAL LABORATORY USE ONLY! MACHEREY-NAGEL products are suited for QUALIFIED PERSONNEL ONLY! MACHEREY-NAGEL products shall in any event only be used wearing adequate PROTECTIVE CLOTHING. For detailed information please refer to the respective Material Safety Data Sheet of the product! MACHEREY-NAGEL products shall exclusively be used in an ADEQUATE TEST ENVIRONMENT. MACHEREY-NAGEL does not assume any responsibility for damages due to improper application of our products in other fields of application. Application on the human body is STRICTLY FORBIDDEN. The respective user is liable for any and all damages resulting from such application.

DNA/RNA/PROTEIN purification products of MACHEREY-NAGEL are suitable for *IN VITRO*-USES ONLY!

ONLY MACHEREY-NAGEL products specially labeled as IVD are also suitable for *IN VITRO*-diagnostic use. Please pay attention to the package of the product. *IN VITRO*-diagnostic products are expressly marked as IVD on the packaging.

IF THERE IS NO IVD SIGN, THE PRODUCT SHALL NOT BE SUITABLE FOR *IN VITRO*-DIAGNOSTIC USE!

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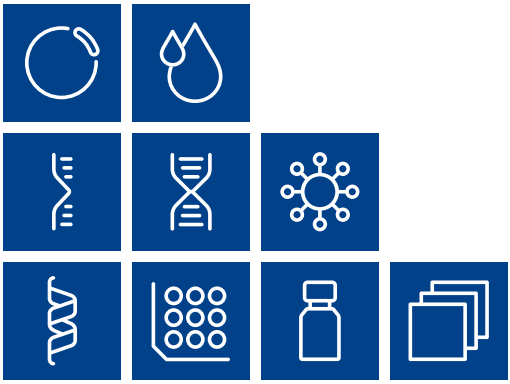

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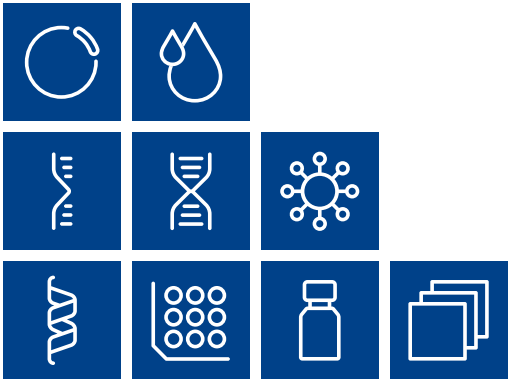

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