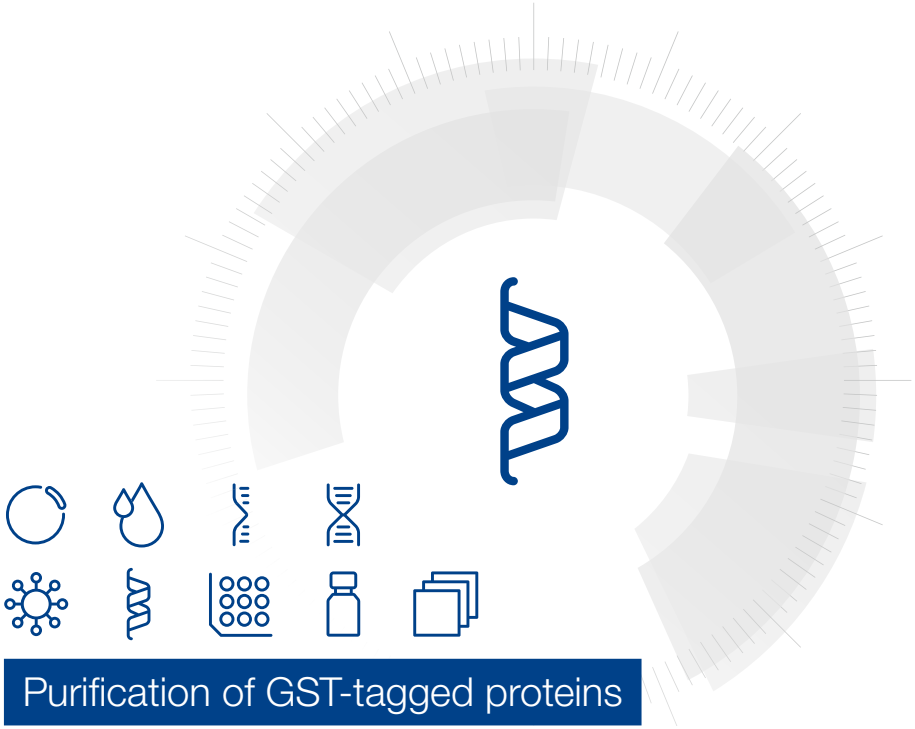
Bioanalysis

MACHEREY-NAGEL

User manual



Purification of GST-tagged proteins

- Protino® GST/4B Columns 1 mL
- Protino® GST/4B Columns 5 mL

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1 Components

1.1 Kit contents and storage

	Protino® GST/4B Columns		
	1 mL	5 mL	
REF	**745500.10**	**745515.1**	**745515.5**
Protino® GST/4B Columns	5 x 1 mL	1 x 5 mL	5 x 5 mL
User manual	1	1	1

Shipping and storage of Protino® GST/4B Columns:

The product is shipped at ambient temperature.

Upon receipt Protino® GST/4B Columns should be **stored at 2–8 °C** (stable until: see package label). Do not freeze.

1.2 Reagents, consumables, and equipment to be supplied by the user

Reagents

- PBS (10 mM Na_2HPO_4, 1.8 mM KH_2PO_4, 2.7 mM KCl, 140 mM NaCl, pH 7.3), see section 2.3
- Elution Buffer (50 mM Tris-HCl, 10 mM glutathione, pH 8), see section 2.3
- Lysozyme (required for cell extract preparation, see section 4.1)

Consumables

- Appropriate centrifuge tubes, collecting tubes

Equipment

- Appropriate centrifuge
- Liquid chromatography system (MPLC, FPLC™, ÄKTAdesign™, etc.), peristaltic pump, or syringe
- If necessary, appropriate adaptors for connecting the Protino® GST/4B Columns to your system of choice. Protino® GST/4B Columns are equipped with 10–32 (1/16”) inlet and outlet ports. With these ports the columns can easily be connected to standard MPLC/FPLC™ systems, (e.g., ÄKTAdesign™). Five adaptor sets are available for connecting the columns to other systems or for using them with a syringe (see Table 1).

Table 1: Adaptor sets

System	Connection via	Adaptor needed	Adaptor Set
Standard FPLC™ system (e.g., ÄKTAdesign™)	10– 32	none	none
FPLC™ system, first generation (Pharmacia)	M6	1 x M6 female to 10– 32 male 1 x 10– 32 female to M6 male	Protino® M6 Adaptor Set REF 745260
MPLC system (e.g., BioLogicTM, BIO-RAD)	1/4" 28	1 x 1/4" 28 female to 10– 32 male 1 x 10– 32 female to 1/4" 28 female	Protino® 1/4–28 Adaptor Set REF 745261
MPLC system (e.g., BioLogicTM, BIO-RAD)	Luer	1 x Luer female to 10– 32 male 1 x 10– 32 female to Luer male	Protino® Luer Adaptor Set REF 745264
Peristaltic pump	1/16" ID tubing inlet	1 x 1/16" ID tubing to 10– 32 male	Protino® Inlet PP Adaptor Set REF 745263
Syringe	Luer inlet	1 x Luer female to 10– 32 male	Protino® Inlet Luer Adaptor REF 745262

1.3 About this user manual

It is recommended to read the instructions of this user manual carefully before use. All technical literature is also available on the internet at ***www.mn-net.com***.

Please contact Technical Service regarding information about changes of the current user manual compared to previous or updated revisions.

2 Introduction

Protino® GST/4B Columns are convenient, ready-to-use FPLC™ columns prepacked with Protino® Glutathione Agarose 4B for rapid purification of GST fusion proteins. The columns can be used with an automated chromatography system, a peristaltic pump, or with a syringe for manual processing.

Protino® GST/4B Columns can be attached directly to liquid chromatography systems (such as ÄKTAdesign™ systems) via standard 10 – 32 fittings. The columns can also be operated with other chromatography systems, with a syringe, or peristaltic pump by using common adapters provided separately by MN (for details see section 1.2 or contact Technical Service Bioanalysis).

The snap-off end of the columns can be reused as stop plug for sealing the outlet of the columns for storage.

2.1 Specifications

Table 2: Specifications Protino® GST/4B Columns

Column bed volume	**1 mL**	**5 mL**
System compatibility	- Automated liquid chromatography systems (MPLC, FPLC™, ÄKTAdesign™, etc.) - Peristaltic pump - Syringe	
Column dimensions	0.7 cm inner diameter x 2.5 cm height	1.6 cm inner diameter x 2.5 cm height
Column body material	Polypropylene	
Column ports	Inlet 10–32 (1/16”) female Outlet 10–32 (1/16”) male	
Matrix	4 % beaded agarose	
Ligand	Glutathione, linked via sulfur atom	
Spacer arm	12 atoms	
Bead size	90 µm	
Binding capacity[1]	~10 mg (recombinant GST)	~50 mg (recombinant GST)
Maximum back pressure	3 bar (0.3 MPa)	

[1] Binding capacity will vary for each GST-tagged protein.

Table 2: Specifications Protino® GST/4B Columns		
Recommended flow rates		
Equilibration	1.0 mL/min	2.5 mL/min
Sample loading[2]	0.2 – 1.0 mL/min	0.5 – 2.0 mL/min
washing and elution	1.0 mL/min	5.0 mL/min
Chemical stability	Protino® GST/4B Columns withstand 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	2–8 °C	
Storage solution	20 % ethanol	

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).

Culture volume requirements are based on the following assumptions:

- Protino® GST / 4B Columns have a binding capacity of ~ 10 mg of recombinant GST per 1 ml bed volume.
- Typically, the expression level of GST-tagged proteins is high, ranging from 10 to 50 mg / liter of *E. coli* culture.
- As a starting point we recommend to use the cell lysate from a 2000–1000 mL *E. coli* culture per 1 mL bed volume.

[2] Slow flow rates are recommended for the loading step to allow maximal binding of the GST-tagged protein.

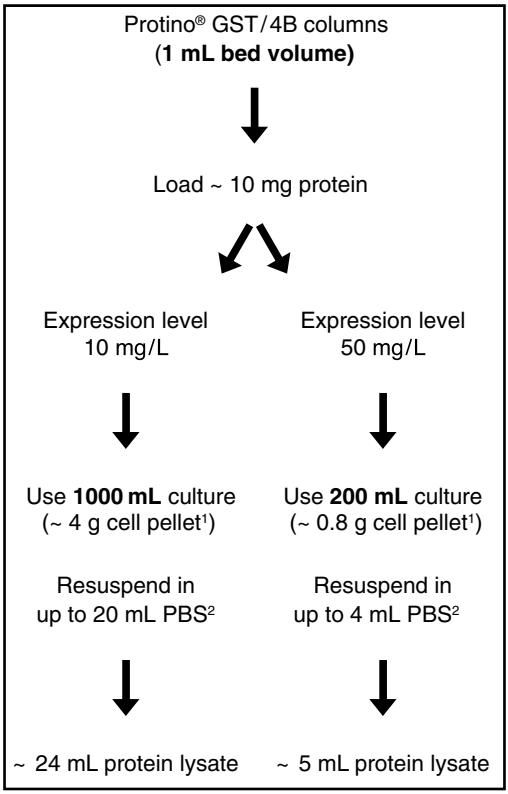

Figure 1 Required culture volumes per 1 ml bed volume

[1] On average, 250 ml of culture will produce approximately 1 g of pelleted, wet cells.
[2] 1 g cells may be lysed in 2–5 mL PBS, see section 4.1.

2.3 Preparation of buffers

PBS (1 liter):

10 mM	Na_2HPO_4	1.780 g	$Na_2HPO_4 \bullet 2\ H_2O$	M_r = 156.01 g/mol
1.8 mM	KH_2PO_4	0.245 g	KH_2PO_4	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

Minimum buffer volumes required for one column run can be calculated according to the following table. Note that additional volumes of PBS and Elution Buffer may be prepared to flush lines and pumps depending on the chromatographic system. As a starting point we recommend to prepare approximately **150 mL of PBS** and **100 mL of Elution Buffer** for the 1 mL column and **300 mL of PBS** and **150 mL of Elution Buffer** for the 5 mL column. Use high-purity chemicals and water for preparing the buffers. For best results, filter buffers through a 0.45 µm filter before use.

	Protino® GST/4B Columns	
	1 mL	**5 mL**
PBS		
5 mL per 1 g of cell pellet for cell extract preparation	20 mL	100 mL
10 mL per 1 mL bed volume for equilibration	10 mL	50 mL
10 mL per 1 mL bed volume for washing	10 mL	50 mL
~ 50 mL of PBS for flushing lines and pump	50 mL	50 mL
Total volume of PBS	90 mL	250 mL
Recommended volume of PBS	**150 mL**	**300 mL**

	Protino® GST/4B Columns	
	1 mL	**5 mL**
Elution Buffer		
10 mL per 1 mL bed volume for equilibration	10 mL	50 mL
~ 50 mL of Elution Buffer for flushing lines and pump	50 mL	50 mL
Total volume of Elution Buffer	60 mL	100 mL
Recommended volume of Elution Buffer	**100 mL**	**150 mL**

3 Safety instructions

When working with the **Protino® GST/4B Columns** 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® GST/4B Columns** kit has not been tested for residual infectious or GMO-material. Therefore, liquid waste must be considered infectious or containing GMO-material and should be handled and discarded according local safety regulations.

3.1 Disposal

Dispose hazardous, infectious, GMO 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

Refer to sections 2.2 for detailed information on culture volume requirements. Prepare PBS as described at section 2.3.

Refer to sections 2.2 for detailed information on culture volume requirements. Prepare PBS as described at section 2.3.

1 Cultivate and harvest cells

- As a starting point we recommend to prepare 200–1000 mL *E. coli* expression culture for the purification of 10 mg of GST-tagged protein using 1 mL bed volume using Protino® GST/4B Columns (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.

4.2 Purification of GST-tagged proteins using Protino® GST/4B Columns

Protino® GST/4B Columns can be operated with liquid chromatography systems (such as ÄKTAdesign™ systems) via standard 10–32 fittings without additional connectors.

Prepare buffers according to section 2.3. For best results, filter buffers through a 0.45 µm filter before use. Clear samples by centrifugation and/or pass them through a 0.45 µm filter.

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

! Prepare Elution Buffer as described at section 2.3. Elution Buffer has to be prepared fresh daily and stored at 4° C.

		Protino® GST/4B columns	
		1 mL	5 mL
1	**Connect column to the chromatography system**		
	• Purge the pump with PBS. Assure that all air is displaced.		
	• Remove the snap off end at the column outlet and save it for further use.		
	• Remove the upper plug from the column.		
	• Start the pump at a flow rate of approximately 0.3 mL/min.		
	• Fill the inlet port of the column with several drops of PBS to remove air to form a positive meniscus.		
	• Insert the fitting “drop-to-drop” into the column port to avoid introducing air bubbles.		
	Note: The snap off end can be reused as a stop plug for sealing the column outlet for storage.		
2	**Column equilibration**		
	• Equilibrate the column with 5–10 column volumes of PBS until the baseline at 280 nm is stable.		
		5–10 mL	**50–100 mL**
	Use a flow rate up to	1 mL/min	**2.5 mL/min**
3	**Binding**		
	• Load the centrifuged or filtered sample onto the column.		
	Use a flow rate up to	0.2–1.0 mL/min	**0.5–2 mL/min**

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

- Collect flow through and analyze, for example 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 flowthrough the column capacity has been exceeded. In this case protein load should be reduced or bed volume should be increased.

4 Washing

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

	10 mL	**50 mL**
Use a flow rate up to	1 mL/min	**5 mL/min**

5 Elution

- Elute the GST-tagged protein with 10 column volumes of Elution Buffer and collect fractions.

	10 mL	**50 mL**
Use a flow rate up to	1 mL/min	**5 mL/min**

- 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.

5 Regeneration and storage

Reuse of Protino® GST/4B Columns 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 column volumes of PBS prior to the next column run.

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

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

If you will not be using the matrix immediately wash with additional 5 column 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® GST/4B Columns. *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® GST/4B Columns have 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 Ordering information

Product	REF	Pack of
Protino® GST/4B Columns 1 ml	745510.5	5 columns
Protino® GST/4B Columns 5 mL	745515.1 745515.5	1 column 5 columns
Protino® Glutathione Agarose 4B	745500.10	10 mL (settled agarose beads)
	745500.100	100 mL (settled agarose beads)
Protino® M6 Adaptor Set	745260	1 set
Protino® 1/4–28 Adaptor Set	745261	1 set
Protino® Luer Adaptor Set	745264	1 set
Protino® Inlet PP Adaptor Set	745263	1 set
Protino® Inlet Luer Adaptor	745262	1

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

6.3 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.

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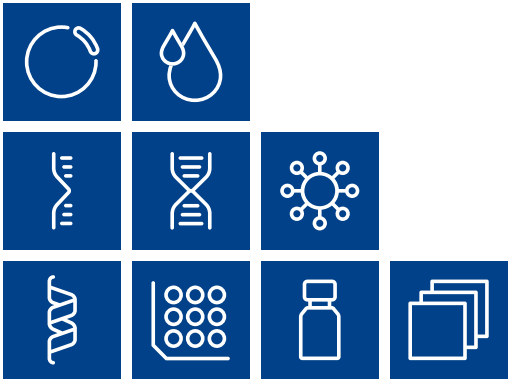

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