

Product Information

Product Name:	Glutathione Beads 4FF
Product Code:	SA010025/SA010100
Size(s):	25ml & 100ml

Product Description:

Glutathione Beads 4FF is an affinity chromatography medium for the fast and efficient one step purification of glutathione S-transferase (GST)-tagged proteins, other GSTs and glutathione-binding proteins. GST-tagged proteins produced through the pGEX series of expression vectors can be purified from the pre-treated bacterial lysate, while using Glutathione Beads 4FF.

GST-tagged proteins binds to the glutathione ligand with high affinity. The coupling of the glutathione ligand to highly cross-linked 4% agarose beads optimized the binding capacity for GST-tagged proteins and other glutathione-binding proteins. Elution of the bound GST-tagged proteins are operated under mild and non-denaturing conditions, with Elution Buffer containing reduced glutathione, to preserve the protein antigenicity and functions. The high flow properties of Glutathione Beads 4FF make it excellent for scaled up purification of GST-tagged protein.

Product Specification:

Chromatography Technique:	Tagged protein purification
Matrix:	Highly cross-linked 4% agarose
Ligand:	Glutathione (coupled via a 12-atom linker arm)
Ligand Concentration:	120 - 320µmol glutathione/ml medium
Binding Capacity:	>10mg GST-fusion protein/ml medium
Particle Size:	45 - 165µm
Maximum Pressure:	3bar (0.3MPa, 43psi)
Maximum Flow Rate*:	450cm/h
pH Stability:	pH 3 - 12
Storage Conditions	2 – 8°C, 1XPBS with 20% ethanol (Do not freeze)

*H₂O at room temperature

Note: The binding between GST and glutathione is dependent on the flow rate. A lower flow rate could increase the binding capacity. The characteristics of the proteins, pH and temperature may also affect the binding capacity. It is important to optimize during sample loading and elution.

Test Items

Specifications

Appearance	White or white-like beads
Volume	50% suspension
Binding Capacity	>10mg GST-tagged protein/ml medium

Operation Protocol

1. Buffer Preparation

Water and chemicals of high purity should be used. It is recommended to filter all buffers through a 0.22µm or 0.45µm filter before usage.

Binding/Wash Buffer: PBS, 140mM NaCl, 2.7mM KCl, 10mM Na₂HPO₄, 1.8mM KH₂PO₄, pH7.4

Elution Buffer: 50mM Tris-HCl, 20mM reduced glutathione, pH8.0

Note: 1-10mM DTT can be added to Binding/Wash Buffer or Elution Buffer

2. Sample Preparation

- 2.1. The sample can be centrifuged and/or filtered through a 0.22µm or 0.45µm filter before applying to the medium
- 2.2. If the sample is too viscous, dilute with Binding Buffer to prevent the column from clogging. Filtration of the sample is not required before batch purification

3. Preparation of Glutathione Beads 4FF

- 3.1. Determine the volume of Glutathione Beads 4FF medium required for the purification process
Note: Glutathione Beads 4FF is delivered as 50% slurry in 20% ethanol
- 3.2. To prepare a 50% slurry for purification, gently shake the bottle of Glutathione Beads 4FF to resuspend the slurry
- 3.3. Use a pipette or measuring cylinder to transfer sufficient slurry to an appropriate tube for use
- 3.4. Sediment the medium through centrifugation at 500 × g for 5 minutes. Decant the supernatant carefully
- 3.5. Wash the Glutathione Beads 4FF with 5ml PBS to each 1ml slurry. Invert the tube to mix
- 3.6. Sediment the medium through centrifugation at 500 × g for 5 minutes. Decant the supernatant carefully
- 3.7. Repeat steps 3.5 and 3.6 once more

4. Batch Purification

- 4.1. Add the cell lysate to the prepared Glutathione Beads 4FF. Incubate at room temperature for 30mins, while using gentle agitation
- 4.2. Use a pipette or cylinder to transfer the mixture to an appropriate tube
- 4.3. Sediment the medium by centrifuging at 500 × g. Decant the supernatant and set it aside to measure the binding efficiency to the medium (i.e. by SDS-PAGE)
- 4.4. Wash the Glutathione Beads 4FF with 5ml PBS each 1ml slurry. Invert the tube to mix
- 4.5. Sediment the medium by centrifuging at 500 × g. Decant the supernatant and set it aside for SDS-PAGE analysis
- 4.6. Repeat step 4.4 and 4.5 twice more. There should be a total of three washes
- 4.7. Elute the bound protein with 0.5ml Elution Buffer per 1ml Glutathione Beads 4FF slurry.

- Incubate at room temperature for 5 - 10 minutes, while using gentle agitation
- 4.8. Sediment the medium through centrifugation at $500 \times g$. Decant the supernatant (eluted protein) carefully
 - 4.9. Repeat step 4.7 and 4.8 twice more. There should be a total of three elutions. Check the eluates for purified proteins and pool

5. Column Packing

- 5.1. Flush the end-piece and adaptor with Packing Buffer to remove the air from the column dead spaces. Ensure that there is no air trapped under the column net
- 5.2. Close the column outlet, while leaving the column net covered with Packing Buffer
- 5.3. Shake the beads container to resuspend the stored beads (Avoid stirring the sedimented medium). To minimise the introduction of air bubbles, pour the slurry down a glass rod while holding it against the column wall.

Note:

If a packing reservoir is in use, fill the remaining column and reservoir with Packing Buffer immediately. Mount the adaptor/lid of the packing reservoir and connect the column to a pump. Avoid trapping air bubbles under the adaptor or in the inlet tubing.

- 5.4. Open the bottom outlet of the column and set the pump to run at the desired flow velocity. The Glutathione Beads 4FF are packed at a constant pressure of approximately 3bar (0.3MPa).
 - i. If the packing equipment does not include a pressure gauge, a packing flow velocity of approximately 400cm/h (10cm bed height, 25°C, low viscosity buffer) should be used
 - ii. If the recommended pressure or flow velocity cannot be obtained, use the maximum flow velocity the pump can deliver to provide a reasonably wellpacked bed. **DO NOT** exceed 75% of the packing flow velocity in subsequent chromatographic procedures
- 5.5. When the bed is stabilized, close the bottom outlet and stop the pump.

Note:

- i. If a packing reservoir is in use, disconnect the reservoir and fit the adaptor to the column.
 - ii. If the column is in use, carefully place the top filter on top of the bed before fitting the adaptor to the column
- 5.6. With the adaptor inlet disconnected, push the adaptor (approximately 2mm) down into the bed. This allows the packing solution to flush the adaptor inlet.
- 5.7. Connect the pump and open the bottom outlet, before resuming packing. The bed should be further compressed and a space will be formed between the bed surface and the adaptor.
- 5.8. Close the bottom outlet. Disconnect the column inlet and lower the adaptor (approximately 2mm) into the bed. Connect the pump. The column is now ready for use

6. Column Purification Protocol

- 6.1. Fill the syringe/pump tubing with Binding Buffer. Remove the stopper and connect the column to the syringe/pump tubing (using the connector provided) in a “drop to drop” motion, to avoid introduction of air into the column. Remove the snap-off end at the

column outlet

- 6.2. Wash the column with 10 column volumes of Binding Buffer
- 6.3. Apply the sample, either through the syringe fitted to the connector or pumping it into the column
- 6.4. Wash the column with 5-10 column volumes of Binding Buffer, until there is an absence of materials in the effluent
- 6.5. Elute with 5 column volumes of Elution Buffer (Volumes of Elution Buffer varies with the ligand-protein interaction).

7. Results Analysis

The eluted fractions can be identified using UV absorbance, SDS-PAGE and western blot

8. Regeneration

Glutathione Beads 4FF can be reused to purify the same protein without requiring regeneration. If a different target GST-fusion protein is applied, the Glutathione Beads 4FF must be regenerated.

- i. **Removal of precipitated or denatured substances:**
 - a. Wash the column with 2 column volumes of 6M guanidine hydrochloride, followed by 5 column volumes of PBS (pH7.4) immediately.
- ii. **Removal of bounded hydrophobic substances:**
 - a. Wash the column with either 3 - 4 column volumes of 70% ethanol or 2 column volumes of 1% Triton™ X-100, followed by 5 column volumes of PBS (pH7.4) immediately.

Storage

For long-term storage, the resin should be stored in 1xPBS containing 20% ethanol at 2 - 8°C.

Troubleshooting Guide

Problems	Possible Causes	Solutions
The yield of the purified GST fusion protein is low or undetectable	The formation of inclusion bodies by fusion protein.	<ul style="list-style-type: none"> • Grow the bacteria at lower temperatures (20 - 30°C). • Reduce the final IPTG concentration to 0.1mM for protein induction. • Reduce the protein induction time. • Prior to the purification, the inclusion body should be properly dissolved and refolded
	The fusion protein does not bind to Glutathione Beads 4FF efficiently	Use the batch method for purification. Incubate the clear solution (by sonication etc.) containing GST-fusion protein with Glutathione Beads 4FF for ≥ 2 hours (such as overnight), before loading the mixture into the column
	There is a lack of an active	Mild sonication condition or other lysis method,



	GST in the fusion protein	such as lysozyme, can be used to prevent denaturation of GST
	The fusion protein has been degraded by protease	Addition of appropriate protease inhibitors, such as PMSF, to the lysis solution and wash solution
	The fusion protein is not efficiently eluted from GST	<ul style="list-style-type: none">• Increase the elution time or the glutathione concentration to $\geq 15\text{mM}$ in the Elution Buffer.• The pH of the Elution Buffer can be adjusted to 8.0 – 9.0, without increasing the glutathione concentration.• Addition of either Triton™ X-100 (final concentration of 0.1%), n-Octylglucoside (final concentration of 2%) or NaCl (final concentration of 0.1 – 0.2 M) to the Elution Buffer.
Multiple bands were observed in the eluted protein	The fusion protein has been degraded by protease	Addition of appropriate protease inhibitors (or inhibitor cocktails), such as PMSF, to the lysis solution and wash solution
	There may be interactions between host proteins, such as chaperonins, and the fusion protein	Addition of DTT (final concentration of 5mM) to the Wash Buffer. Prior to purification, incubate the recombinant protein solution in chaperonin buffer (2mM ATP, 10mM MgSO ₄ , 50mM Tris-HCl) at 37°C for 10 minutes
	Over-sonication will cause some protein to bind to the fusion protein	Mild sonication condition or other lysis method, such as lysozyme, can be used
	Some protein will bind non-specifically to the fusion protein or beads	Optimize the wash conditions. Detergents, such as 1% Triton™ X-100, 1% Tween-20, 0.03% SDS and 0.1% NP-40, may be used to reduce non-specific binding. Salt concentration can also be titrated and optimized