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ab270237 Glutathione Affinity Resin - Amintra

A product of Expedeon, an Abcam company

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For simple, rapid purification of Glutathione-S-transferase-tagged recombinant proteins and glutathione binding proteins from bacterial, yeast, insect and mammalian cell cultures.

This product is for research use only and is not intended for diagnostic use.

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1. Overview

Glutathione Affinity Resin - Amintra (ab270237) is an affinity chromatography medium for easy and rapid one step purification of Glutathione-S-transferase (GST)-tagged recombinant proteins produced using the pGEX series of expression vectors, other glutathione-S-transferases and glutathione binding proteins from bacteria, yeast, insect and mammalian cultures.

GST-tagged proteins can be purified directly from pre-treated bacterial lysates using Glutathione Affinity Resin - Amintra. The tagged proteins are eluted under mild, non-denaturing conditions that preserve protein antigenicity and function.

The glutathione ligand is coupled to highly cross-linked 4% agarose beads. The coupling is optimized to give high binding capacity for GST-tagged proteins and other glutathione binding proteins. Glutathione Affinity Resin - Amintra can purify GST-tagged proteins under high flow rate. Its high flow properties make it excellent for scale-up.

2. Materials Supplied and Storage

Store kit at +4°C immediately on receipt. **Do not freeze or store the resin at room temperature. Freezing the suspension will damage the agarose beads.** The resin is pre-swollen and defined. It is formulated as a 50% suspension in 20% ethanol.

Item	Quantity			Storage temperature
Glutathione Affinity Resin - Amintra	10 mL	25 mL	100 mL	+4°C

Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- Binding buffer (see buffer selection Section 4.3)
- Wash buffer (see buffer selection Section 4.3)
- Elution buffer (see buffer selection Section 4.3)

3. Technical Considerations

3.1 Chemical compatibility:

All resins are susceptible to oxidative agents. Avoid high temperatures. The resin is resistant to short exposure to organic solvents (e.g. 70 % ethanol) or denaturants (e.g. 6M Guanidine hydrochloride) and are stable in all aqueous buffers commonly used for cleaning-in-place e.g. 1 M NaOH, 0.01 M HCl.

3.2 Characterization of the Resin:

Supporting matrix	4% highly crosslinked agarose resin
Bead size range	45-165 µm
Recommended working pH	pH 3.0-12.0
Typical binding capacity	> 10 mg GST-tagged protein/mL resin
Recommended Flow rate*	100 - 300 cm/h
Maximum Flow rate	450 cm/h
Maximum pressure	3 bar, 43 psi
Chemical stability	High
Solubility in water	Insoluble
Ligand	The glutathione ligand is coupled via a 12-atom linker
Ligand concentration	120-320 µmol glutathione/mL medium

** Binding of GST to glutathione is flow dependent and lower flow rates often increase the binding capacity. This is important during sample loading and elution. Protein characteristics, pH and temperature may also affect the binding capacity.*

3.3 Recommended buffers:

Water and chemicals used for buffer preparation should be of high purity. We recommend filtering the buffers by passing them through a 0.22 µm or 0.45 µm filter before use.

Binding buffer: 10 mM sodium phosphate, 140 mM NaCl, 2.7 mM KCl, 1.8 mM KH_2PO_4, (PBS); pH 7.4

Wash buffer: 10 mM sodium phosphate, 140 mM NaCl, 2.7 mM KCl, 1.8 mM KH_2PO_4, (PBS); pH 7.4

Elution buffer: 50 mM Tris-HCl, 20 mM glutathione, pH 8.0.

Δ Note: *Binding of GST-tagged protein glutathione resins is not efficient at pH below 6.5 or pH above 8.0. Please note that most GST-tagged proteins will elute from the column with some reduced glutathione.*

Δ Note: *1-10 mM DTT can be added to all the buffers.*

4. Assay Procedure

4.1 Sample preparation:

- The sample should be centrifuged and/or filtered through a 0.22 µm or 0.45 µm filter before it is applied to the medium.

- If the sample is too viscous, dilute it with binding buffer to prevent clogging the column. It is not necessary to filter the sample before performing batch purification.

4.2 Batch purification:

Resin preparation:

4.2.1 Determine the medium volume of Glutathione Affinity Resin - Amintra that is required for your purification.

*Δ **Note:** Glutathione Affinity Resin - Amintra is 50% slurry with 20% ethanol.*

4.2.2 To prepare a 50% slurry for your purification, gently shake the bottle of Glutathione Affinity Resin - Amintra to resuspend the slurry.

4.2.3 Use a pipette or measuring cylinder to remove sufficient slurry for use and transfer to an appropriate tube.

4.2.4 Sediment the medium by centrifugation at 500 x *g* for 5 minutes. Carefully decant the supernatant.

4.2.5 Wash the Glutathione Affinity Resin - Amintra by adding 5 mL PBS to each 1 mL slurry. Invert to mix.

4.2.6 Sediment the medium by centrifugation at 500 x *g* for 5 minutes. Carefully decant the supernatant.

4.2.7 Repeat steps 5.2.5 and 5.2.6 one more time.

Batch Purification protocol:

4.2.8 Add the cell lysate to the prepared Glutathione Affinity Resin - Amintra and incubate for at least 30 minutes at room temperature. Use gentle agitation such as end-over-end rotation.

4.2.9 Use a pipette or cylinder to transfer the mixture to an appropriate container/tube.

4.2.10 Sediment the medium by centrifugation at 500 x *g* for 5 minutes. Carefully decant the flow-through and save it for measuring the binding efficiency to the medium by for example SDS-PAGE. We recommend our RunBlue™ range of precast gels as well as our new GST-Tag Protein Expression Check Kit (ab270052) expression validation lateral flow assay.

4.2.11 Wash the Glutathione Affinity Resin - Amintra by adding 5 mL PBS to each 1 mL slurry. Invert to mix.

4.2.12 Sediment the medium by centrifugation at 500 x g for 5 minutes. Carefully decant the supernatant (= wash) and save it for SDS-PAGE analysis.

4.2.13 Repeat steps 5.2.11 and 5.2.12 twice for a total of three washes.

4.2.14 Elute the bound protein by adding 0.5 mL 50 mM Tris-HCl, 20 mM reduced glutathione, pH 8.0 per 1 mL slurry of Glutathione Affinity Resin - Amintra. Incubate at room temperature for 5–10 minutes using gentle agitation such as end-over-end rotation.

4.2.15 Sediment the medium by centrifugation at 500 x *g* for 5 minutes. Carefully decant the supernatant (= eluted protein).

4.2.16 Repeat steps 5.2.14 and 5.2.15 twice for a total of three elutions. Check the three eluates separately for purified protein and pool according to the results.

4.3 Column purification:

Column packing:

4.3.1 Remove air from the column dead spaces by flushing the end-piece and adapter with packing buffer. Make sure no air has been trapped under the column net.

4.3.2 Close the column outlet leaving the net covered with packing buffer.

4.3.3 Resuspend the beads stored in its container by shaking (avoid stirring the sedimented medium). Pouring the slurry down a glass rod held against the column wall will minimize the introduction of air bubbles. If using a packing reservoir, immediately fill the remainder of the column and reservoir

with packing buffer. Mount the adapter or lid of the packing reservoir and connect the column to a pump. Avoid trapping air bubbles under the adapter or in the inlet tubing.

4.3.4 Open the bottom outlet of the column and set the pump to run at the desired flow velocity. Ideally, Glutathione Affinity Resin - Amintra is packed at a constant pressure of approximately 0.3 bar (0.3 MPa). If the packing equipment does not include a pressure gauge, use a packing flow velocity of approximately 400 cm/h (10 cm bed height, 25°C, low viscosity buffer). If the recommended pressure or flow velocity cannot be obtained, use the maximum flow velocity the pump can deliver. This should also give a reasonable well-packed bed. Do not exceed 75% of the packing flow velocity in subsequent chromatographic procedures.

4.3.5 When the bed has stabilized, close the bottom outlet and stop the pump. If using a packing reservoir, disconnect the reservoir and fit the adapter to the column. If using the column, carefully place the top filter on top of the bed before fitting the adapter.

4.3.6 With the adapter inlet disconnected, push the adapter down, approximately 2 mm into the bed, allowing the packing solution to flush the adapter inlet.

4.3.7 Connect the pump, open the bottom outlet and continue packing. The bed will be further compressed at this point and a space will be formed between the bed surface and the adapter.

4.3.8 Close the bottom outlet. Disconnect the column inlet and lower the adapter approximately 2 mm into the bed. Connect the pump. The column is now ready to use.

Column Purification protocol:

4.3.9 Fill the syringe or pump tubing with binding buffer. Remove the stopper and connect the column to the syringe (with the provided connector), or pump tubing, "drop to drop" to avoid introducing air into the column. Remove the snap-off end at the column outlet.

4.3.10 Wash the column with 10 column volumes of binding buffer.

4.3.11 Apply the sample, using a syringe fitted to the connector or by pumping it onto the column.

4.3.12 Wash with 5 to 10 column volumes of binding buffer or until no material appears in the effluent.

4.3.13 Elute with 5 column volumes of elution buffer. Other volumes may be required if the interaction is difficult to break.

4.3.14 The eluate should be collected for further analysis. Always check the protein content of each fraction before pooling to avoid unnecessary dilution of the purified target protein.

4.4 Analysis:

Identify the fractions using UV absorbance, SDS-PAGE, or western blot. We recommend our RunBlue™ range of precast gels as well as our new GST-Tag Protein Expression Check Kit (ab270052) expression validation lateral flow assay.

4.5 Regeneration:

Glutathione Affinity Resin - Amintra can be reused to purify the same protein three times without regeneration. If the target GST-fusion protein is different, however, the Glutathione Affinity Resin - Amintra must be regenerated using the following protocol:

4.5.1 For removal of precipitated or denatured substances: Wash with two column volumes of 6 M guanidine hydrochloride, immediately followed by five column volumes of PBS, pH 7.4.

4.5.2 For removal of hydrophobically bound substances: Wash with three to four column volumes of 70% ethanol or two column volumes of 1% Triton™ X-100, immediately followed by five column volumes of PBS, pH 7.4.

4.6 Resin storage:

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

5. Frequently asked questions

5.1 What is the shelf-life of Glutathione Affinity Resin - Amintra?

The resin is guaranteed for 12 months after the date of manufacture provided they are stored at 2-8°C.

5.2 Do I need to filter the buffers prepared in my laboratory?

It is good laboratory practice to filter all buffers using a 0.45 micron filter.

5.3 How should I prepare my buffer the Glutathione Affinity Resin - Amintra?

Elution buffers, in particular, should be prepared fresh before use. Reduced glutathione gradually becomes oxidized in solution. It is recommended that you add fresh reduced glutathione and/or other reducing agents to the elution buffer just prior to use. You will need need to re-adjust pH of the buffer system after addition of reduced

glutathione.

5.4 Should I add DTT to the lysis buffer?

Concentrations less than or equal to 10 mM DTT can be used with this resin.

5.5 Should I be concerned if the resin partially dried out during the chromatographic steps?

The resin is robust. Partially dried resin rehydrates rapidly. There are no adverse effects upon the performance of the resin.

5.6 Do I need to remove the GST-tag from the recombinant protein?

Typically a protease cleavage site is engineered between the GST-tag and the target protein. The GST-tag can be cleaved on the column or in solution after elution. Cleavage of the GST tagged protein on the column eliminates the need to separate the protein from the GST tag at a later date as the GST tag remains bound to the column.

5.7 What are the endotoxin levels in the resin?

The endotoxin levels are below the detection levels.

5.8 Do you have any data regarding ligand leakage?

Ligand leakage, at the point of coupling, is negligible.

5.9 Can I regenerate the resin?

Glutathione Affinity Resin - Amintra can be reused to purify the same protein three times without regeneration. If the target GST-fusion protein is different, however, the Glutathione Affinity Resin - Amintra must be regenerated using the following protocol: For removal of precipitated or denatured substances: *Wash with 2 column volumes of 6 M guanidine hydrochloride, immediately followed by 5 column volumes of PBS, pH 7.4.* For removal of hydrophobically bound substances: *Wash with 3-4 column volumes of 70% ethanol or 2 column volumes of 1% Triton™ X-100, immediately followed by 5 column volumes of PBS, pH 7.4.*

5.10 Can I re-use the resin?

The resin can be re-used. Re-use does depend on the properties of your target protein. You may observe that flow rates slow down in successive bind-washelute cycles as more samples are progressively loaded on to the columns. In addition, if the resin is not regenerated, binding capacity may be reduced.

6. Troubleshooting

6.1 Bubbles or cracks appear in the resin bed

- The resin has been stored at a cool temperature and then rapidly warmed up. Amintra resins should be warmed slowly to room temperature before use.

6.2 The sample does not flow easily through the resin

- The resin is clogged with particulates. Pre-filter the sample just before loading it on to the resin.
- If the resin is not stored at 2-8 °C, or they have been used more than once and stored in the absence of a bacteriostat, microbial growth in the column may restrict flow through the resin.

6.3 No elution of the target protein is observed from the resin

- The elution conditions are too mild to desorb the target protein. Use a higher concentration of reduced glutathione (GSH). Use fresh reduced glutathione.
- Increase the pH of the elution buffer. Increasing the elution buffer to pH 8-9 may assist elution of the GST-tagged protein.
- Ensure that there are no denaturants in the sample and buffers as they may interfere with the binding.
- The protein may have precipitated in the column.
- The cell disruption method may have liberated proteolytic activities. Purify the protein under denaturing conditions if you do not need to purify an active protein.

6.4 The recovery of target protein is low

- Ensure that the resin bed volume is proportionate to the level of expressed GST-tagged protein. The target protein may pass through into the sample wash if the capacity of the resin plug is insufficient for the level of expressed protein.
- Add 1-10 mM DTT to increase binding of GST-tagged proteins to the resin.
- Confirm levels of target protein by immunoassay. This will help determine if your cell disruption methods have been successful.

- The target protein may contain hydrophobic stretches which could have been toxic to the host.
- Add further Proteoloc Protease Inhibitor Cocktail to the buffers as the full-length protein may have been degraded by hydrolytic enzymes. Alternatively, reduce the time of expression, lower the temperature at which the protein is exposed or use special E.coli strains devoid of proteases. Remember to remove the serine protease inhibitors before cleavage with TEV-Express or 3C-Express.

6.5 Poor resolution of the target protein

- The sample volume or concentration may be too large for the capacity of the resin plug. In this case, reduce the sample load or sample volume.
- The sample may also need to be filtered carefully.

6.6 The target protein elutes at an unexpected position

- There may be an ionic interaction between the protein and the resin. You should maintain the ionic strength above 0.1 M.
- There may be hydrophobic interactions between the sample and the resin. In this instance, reduce the salt concentration.
- Co-purification of contaminants may occur if both the expressed protein and the contaminant have similar affinities for the matrix. In this case, a further chromatographic method such as gel filtration or ion exchange chromatography is recommended.

6.7 The elution profile cannot be reproduced

- The nature of the sample may have altered so it may be important to prepare a fresh sample. The GST-tag may have been removed by proteases. Work at 2-8°C and add Proteoloc Protease Inhibitor Cocktail to the lysis buffer.
- The sample load may be different from the original sample load. It is advisable to keep all these parameters constant.
- Proteins or lipids may have precipitated in the resin bed. Use elution conditions, which stabilize sample.
- The buffer pH and ionic strength are incorrect and new buffers will need to be prepared.

Technical Support

For all technical or commercial enquiries please go to:

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