

# Amintra

## Affinity Resins

# Glutathione

**Affinity Resin**  
**Technical Data and Instruction Manual**



**expedeon**  
PROTEIN SOLUTIONS

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## **Introduction**

Expededon's Glutathione resin is designed for rapid one-step purification of glutathione S-transferase-tagged proteins from bacteria, yeast, insect and mammalian cultures. Glutathione is covalently coupled to a high crosslinked agarose matrix that provides a high chemical and physical stability and excellent flow rates. The resin is made in the particle size range of 40-165  $\mu\text{m}$  and has a dynamic binding capacity of 10 mg recombinant GST/ml resin. Amintra Glutathione is stable with all commonly used buffers and reagents including 0.1M NaOH and organic solvents. Removal of the GST tag can be performed whilst the fusion protein is bound to the column or in solution after elution.

## **Storage**

Store the Amintra Glutathione resin at 2-8 °C. 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 in 20 % ethanol. Amintra Glutathione resin is stable for up to 3 years at 2-8 °C from the date of manufacture. For expiry date please see product.

## **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.

## Specifications

Supporting matrix:	4% highly crosslinked agarose resin
Bead size range:	40 -165 $\mu\text{m}$
Recommended working pH:	pH 4.0-10.0
Typical binding capacity:	$\sim$ 10 mg GST-tagged protein/ml resin
Recommended Flow rate	1-2 ml/min/cm <sup>2</sup>
Maximum Flow rate	4 ml/min/cm <sup>2</sup>
Maximum pressure	3 bar
Chemical stability:	High
Solubility in water:	Insoluble

## Disclaimer

This product is for research use only and is not intended for use in clinical diagnosis. No claims beyond replacement of unacceptable material or refund of purchase price shall be allowed.

## Recommended Buffers

Equilibration buffer    20mM sodium phosphate, 0.15M NaCl, pH 7.2\*

Wash buffer                20mM sodium phosphate, 0.15M NaCl, pH 7.2\*

Elution buffer            20mM sodium phosphate, 0.15M NaCl, 5-100mM glutathione, pH 7.2\*

\*Binding of GST-tagged protein glutathione resins is not efficient at pH below 6.5 or pH above 8.0. Alternative buffers systems that can be used include 50mM Tris pH 8.0 and 20 mM HEPES. Please note that most GST-tagged proteins will elute from the column with 5-20mM reduced glutathione.

If you wish to pack a column then gently shake the bottle to form uniform slurry. It is often preferable to de-gas the resin slurry. Pour or pipette the resin slurry into a glass or plastic column with the column outlet slightly open. Add 5-10 column volumes of distilled water to wash the resin and to ensure that the resin is packed well. Close the column outlet valve. The column is now ready for pre-equilibration with binding buffer.

## **Protein Purification Protocol**

### *Pre-equilibration:*

Equilibrate the resin with 5 column volumes of binding buffer.

### *Sample loading:*

Load an appropriate amount of 0.45  $\mu$ m filtered cleared lysate on to column. Or if possible, mix the resin with the cleared lysate and let the resin settle for 30 minutes at room temperature or at 2-8 °C. The binding capacity of the resin is approximately 10 mg GST-tagged protein/ml sedimented resin. Collect the sample flowthrough for further analysis.

### *Washing:*

Wash the column with 10-25 column volumes of wash buffer. Collect the washes for further analysis to ensure that all unbound protein is removed.

*Elution:*

Apply 10-15 column volumes elution buffer to the resin and collect appropriate fractions sizes (e.g 1CV) for further analysis.

Alternatively you can apply 2 column volumes of elution buffer to the resin, mix and allow the resin to settle. Following this, collect the supernatant. Repeat this process at least 5 times.

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.

## **Questions and Answers**

*1. What is the shelf-life of Amintra Glutathione resin?*

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

*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.

*3. How should I prepare my buffer the Amintra Glutathione resin?*

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 to re-adjust pH of the buffer system after addition of reduced glutathione.

*4. How should I prepare my sample the Amintra Glutathione resin?*

It is recommended that all samples are filtered to at least 0.45  $\mu\text{m}$  pore size.

*5. Should I add  $\beta$ -mercaptoethanol or DTT to the lysis buffer?*

Concentrations less than or equal to 10 mM  $\beta$ -mercaptoethanol or DTT can be used with this resin.

*6. 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.

*7. 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. When protein precipitation is observed during cleavage Expedeon's Stabil-PAC (# STP) can be used to maintain protein solubility.

*8. What are the endotoxin levels in the resin?*

The endotoxin levels are below the detection levels.

*9. Do you have any data regarding ligand leakage?*

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

10. Can I regenerate the resin?

Regenerate the column by passing 5 CVs of 0.1 M Tris/HCl buffer, 0.5 M NaCl, pH 8.5, then 5 CVs of 0.1 M sodium acetate buffer, 0.5 M NaCl, pH 4.5 and finally 5 CVs of either 1X PBS buffer pH 7.2 or running buffer. Alternatively, use 3 CVs of either 6M Guanidine HCl, 70% ethanol or 0.1M NaOH for cleaning followed with at least 5 CVs of either 1X PBS buffer pH 7.2 or running buffer.

*11. 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-wash-elute cycles as more samples are progressively loaded on to the columns. In addition, if the resin is not regenerated, binding capacity may be reduced.

## Troubleshooting assistant

*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.

*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.

*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. Use StabilPAC (# STP) to enhance protein solubility.

- 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.

*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 protease inhibitors 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 Thrombin or Factor Xa.

*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.

*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 and use Stabil-PAC (# STP) to reduce non-specific binding.
- 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.

*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 a 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 the sample.
- The buffer pH and ionic strength are incorrect and new buffers will need to be prepared.

## **Ordering Information**

Cat #	Description
AGS0005	Amintra Glutathione Resin- 5ml
AGS0025	Amintra Glutathione Resin - 25ml
AGS0100	Amintra Glutathione Resin - 100ml

## Contact Information

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