

FACT: The most competitively-priced premium Glu agarose resin in the market

Technical Data and Operating Instructions

generon

Glutathione Superflow (SuperGlu) Resin

1. Optimized for high-performance purification of GST-tagged proteins and GST pull-down assays.
2. Negligible ligand leakage.
3. Typical binding capacity of 10 mg/ml resin
4. Higher flow rates than Sepharose resin.
5. Can withstand pressures up to **120-140 psi**.
6. Compatible with a wide range of reducing agents, denaturants etc.
7. Mild elution of GST-tagged proteins with reduced glutathione.
8. Supplied in 10 ml, 25 ml or 100 ml quantities.
9. cGMP manufacturing.

The Product

SuperGlu (Glutathione Superflow) 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 Superflow matrix that provides a high chemical and physical stability and excellent flow rates. SuperGlu is made in the particle size range of 60-160 μm and has a dynamic binding capacity of 10 mg recombinant GST/ml resin. SuperGlu is stable with all commonly used buffers and reagents including 0.1M NaOH and organic solvents. Removal of the GST tag (a naturally occurring protein with a molecular weight of 26 kDa) can be undertaken while bound to the column or in solution after elution using typical site-specific proteases such as PreScission™ Protease, Factor Xa or Thrombin.

Storage conditions

Store the SuperGlu 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 de-fined. It is transported in 20 % ethanol. Generon's resin is stable for up to 3 years at 2-8 °C from the date of manufacture. The expiry date is recorded clearly on the label of the bottle.



Specifications

Agarose concentration:	6% cross-linked agarose
Bead Size:	60-160 μm
Ligand density:	32 $\mu\text{mole/ml}$ gel
Support:	Superflow 6 matrix
Binding capacity:	10 mg GST-tagged protein/ml resin
Max. pressure:	120-140 psi
Max. linear flow rate:	1,400 cm/hr (determined using a 1.5 x 13 cm column)
Max. flow rate:	40 ml/min (determined using a 1.5 X 15 cm column)
Exclusion limit:	6X10 ⁶
pH stability:	pH 3-12
Cleaning:	0.1M NaOH for 30 min
Slurry concentration:	57 %
Antimicrobial agent:	20% ethanol
Storage condition:	20% ethanol at 2-8 °C

Chemical compatibility of the SuperGlu resin

All resins are susceptible to oxidative agents. Avoid high temperatures. The resin is resistant to short exposure to denaturants (e.g. 6 M guanidine HCl), organic solvents (e.g. 70 % ethanol) and is stable in all aqueous buffers commonly used for cleaning-in-place e.g. 0.1 M NaOH, 0.1 M HCl.

Technical support

Contact the Generon technical support and sales centre for assistance

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Protein Purification Protocol

Reagents:

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 to Super Glu or any other GST-binding resin is not efficient at pH below 6.5 and pH above 8.0. 50 mM Tris buffer pH 8.0 or 20 mM HEPES buffer are alternative buffer systems that can be used. Please note that most users elute their GST-tagged proteins with 5-20 mM reduced glutathione. We recommend that all buffers including the lysis buffer contain between 1-5 mM DTT to ensure that the Glutathione is in a reduced state. A 1 M DTT stock in laboratory water can be prepared, filtered through a 0.2 µm filter and aliquoted prior to storage in a -20C freezer until use.

Resin Packing

Gently shake the bottle to form a 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 Glu-Superflow 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.

PROTOCOL USING GRAVITY FLOW OR A PERISTALTIC PUMP

Pre-equilibration:

Equilibrate the Glu-Superflow column with 5 column volumes of binding buffer.

Sample loading:

Load an appropriate amount of 0.45 µm filtered cleared lysate on to the Glu-Superflow column. If possible, mix the resin for 30 minutes at room temperature or at 2-8 °C with the cleared lysate. Please note that the binding capacity of the resin is approximately 10 mg GST-tagged protein/ml sedimented resin. Collect the sample wash for further analyses.

Washing:

Wash the column with 10-25 column volumes of wash buffer. The washes should be collected for further analyses to ensure that all unbound protein is removed.

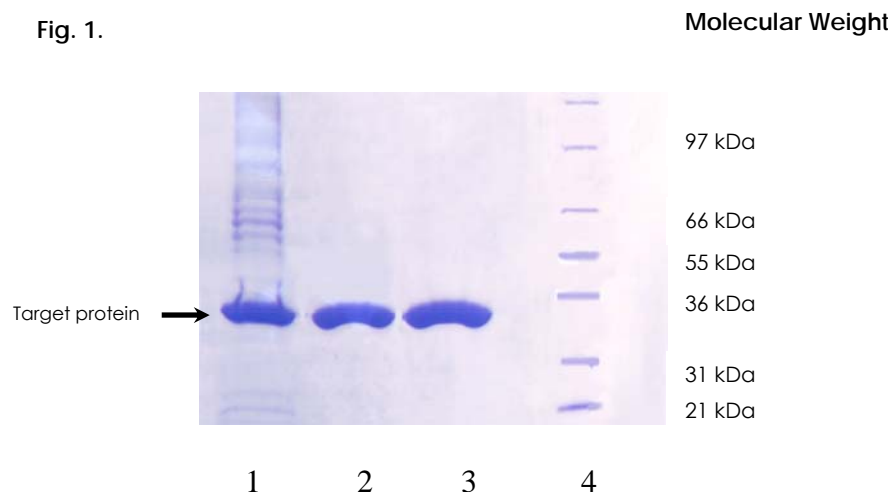
Elution:

Elute the bound GST-tagged protein into fresh tubes with a minimum 5 x 2 column volumes (e.g. 10-15 CVs) of elution buffer. The eluate should be collected for further analyses. Check the protein content of each eluted fraction before pooling them. Otherwise, you risk diluting a concentrated, purified sample.

DESALTING AND CONCENTRATING THE PURIFIED PROTEIN

Glutathione should be removed by diafiltration using Vivaspin ultrafiltration concentrators supplied by Generon, rapid dialysis against an appropriate buffer for your downstream application or gel filtration.

Figure 1 showing high quality purification of a GST-tagged protein from *E. coli* cell lysate.



Lane 1: *E. coli* expressing GST lysate

Lane 2: Eluted GST-tagged protein from the main competitor G

Lane 3: Eluted GST-tagged protein from **SuperGlu**

Lane 4: Molecular weight standards

QUESTIONS AND ANSWERS

1. What is the shelf-life of the SuperGlu resin?

The resin is guaranteed for 3 years after the date of manufacture provided it is 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 µm filter.

3. How should I prepare my buffers?

Elution buffers, in particular, should be prepared fresh before use. Reduced glutathione gradually becomes oxidized in solution. In this instance, we recommend that you add fresh reduced glutathione and/or 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?

Many chromatographic procedures demand that the sample is pre-conditioned prior to loading. We recommend that all samples are filtered to at least 0.45 µm pore size.

5. Should I add β-mercaptoethanol or DTT to the lysis buffer?

Concentrations less than or equal to 10 mM β-mercaptoethanol or DTT can be used with Glu-Superflow resin.

6. Can I use Tris buffer?

Yes, try 50 mM Tris-HCl, pH 8.0.

7. Should I be concerned if the resin partially dries 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.

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

Normally, a protease cleavage site e.g. Factor Xa Protease 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.

9. What are the endotoxin levels in the resin?

The endotoxin levels are below the detection levels.

10. Do you have any data regarding ligand leakage?

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

11. Can I regenerate the SuperGlu 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.

TROUBLESHOOTING ASSISTANT

Bubbles or cracks appear in the resin bed

- The resin has been stored at a cool temperature and then rapidly warmed up. The resin 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 SuperGlu resin.
- If the resin is not stored at 2-8 °C, or it has been used more than once and stored in the absence of a bacteriostat, microbial growth 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. Try 50 mM reduced glutathione in fresh elution buffer.
- Ensure that there are no denaturants in the sample which will interfere with binding of the target protein to the resin.
- Use a non-ionic detergent e.g. 0.1 % Triton X-100 to improve the elution of some GST-tagged proteins.
- Increase the elution buffer pH. Increasing the elution buffer to pH 8-9 may assist elution of the GST-tagged protein.
- The protein may have precipitated in the column.
- The cell disruption method may have liberated proteolytic activities.

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 bed is insufficient for the level of expressed protein.
- Add 1-10 mM DTT to increase and ensure consistent binding of GST-tagged proteins to the SuperGlu 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 bacterium, *E.coli*.
- Add further protease inhibitors eg 1 mM PMSF to the lysis buffers as the full-length protein may have been degraded by hydrolytic enzymes. Remember to remove the serine protease inhibitors before cleavage with Thrombin or Factor Xa. Alternatively, reduce the time of expression, lower the temperature at which the protein is exposed or use special *E.coli* strains devoid of proteases.

Poor resolution of the target protein

- The sample volume or concentration may be too large for the capacity of the resin bed. 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 SuperGlu 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 add suitable detergents or organic solvents.
- 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 and 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

Order Code	Description	Price (£)
SuperGlu 2	Glutathione Superflow (2 ml)	£30.00
SuperGlu 10	Glutathione Superflow (10 ml)	£120.00
SuperGlu 25	Glutathione Superflow (25 ml)	£275.00
SuperGlu 100	Glutathione Superflow (100 ml)	£900.00

SuperGlu resin is available in bulk pack sizes from Generon for industrial applications (up to 5 L quantities). Please contact Generon for pricing information.

Other products sold by Generon

Generon is an authorized UK distributor for Sartorius, Pro-Chem, Glycon, Fermentas and Nalgene. Please visit the Generon website (www.generon.co.uk) for information about our rapid and convenient Protein A and G antibody and Ni-metal chelate 6xHis-tagged protein purification kits. We also supply premium Ni-Sepharose (IDA) and Ni-Superflow (Tetradentate) resin in 25 ml and 100 ml quantities:

Order Code	Description	Price (£)
PC-MC25	Ni-MC resin (25 ml)	£125.00
PC-MC100	Ni-MC resin (100 ml)	£380.00

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A Drug Master File is on file with regulatory agencies in the US and Europe. Superflow™ is a registered trademark.