

Product Information

ANTI-GLUTATHIONE-S-TRANSFERASE (GST) AGAROSE CONJUGATE

Product Number **A 8580**

Product Description

Anti-Glutathione-S-Transferase (GST) is developed in rabbit using repeated injections of recombinant GST from *Schistosoma japonicum* expressed in *E. coli* as the immunogen. Whole antiserum is fractionated and then further purified by ion-exchange chromatography to provide the IgG fraction of antiserum that is essentially free of other rabbit serum proteins. The purified antibody fraction is then coupled to cyanogen bromide-activated agarose at 7 to 8 mg antibody per ml bed volume. Anti-Glutathione-S-Transferase (GST) Agarose conjugate may be used for the purification of GST.

Reagent

Anti-Glutathione-S-Transferase (GST) Agarose conjugate is provided as a 1:1 suspension in 0.01 M phosphate buffered saline pH 7.4, containing 15 mM sodium azide.

Precautions and Disclaimer

Due to the sodium azide content a material safety data sheet (MSDS) for this product has been sent to the attention of the safety officer of your institution. Consult the MSDS for information regarding hazardous and safe handling practices.

Storage/Stability

For continuous use and extended storage, store at 2 °C to 8 °C in buffer containing 15 mM sodium azide.

Do not freeze.

Product Profile

1 ml of settled Anti-Glutathione-S-Transferase (GST) Agarose conjugate has a binding capacity of at least 100 µg of GST.

Procedure

Purification of GST

To prevent clogging the column, highly viscous samples containing chromosomal DNA or RNA should be sonicated or treated with nuclease to reduce the viscosity, and cellular debris and particulate matter must be removed by centrifugation or filtration. Perform all steps at room temperature.

- A. Column Set Up
 1. Place the empty chromatography column on a firm support.
 2. Rinse the column with PBS (Product No. D 8537).
 3. Allow the buffer to drain from the column and leave residual PBS in the column to aid in packing the Anti-GST Agarose conjugate.
- B. Packing the column
 1. Thoroughly suspend the vial of Anti-GST Agarose conjugate to make a uniform suspension of the beads.
 2. Immediately transfer the desired volume to the column. Allow the agarose bed to settle.
Do not let the agarose bed run dry!
- C. Washing the column

Wash the resin with three sequential 5 ml aliquots of glycine-HCl pH 2.5 (or 3 M NaSCN) followed by three sequential 5 ml aliquots of PBS. Avoid disturbing the agarose bed while loading.
- D. Binding GST to the column
 1. Load the sample on the column under gravity flow.
Note: Depending upon the sample and the flow rate, not all of the protein may bind. Multiple passes over the column or closing the loaded column and incubating it on a rotator for about one hour, may improve the binding efficiency.
 2. Collect the "flow through" of unbound protein.
 3. Wash the column with PBS until the OD₂₈₀ ≤ 0.01.
- E. Elution of GST

Elute the bound GST from the column with 10 x 1 ml aliquots of 0.1 M glycine-HCl at pH 2.5 into vials containing 30 to 50 µl of 1 M Tris-HCl buffer pH 8.0 for neutralization.

Notes: Occasionally low pH may cause the eluted protein to aggregate. In such cases choose an alternative buffer for elution, for example 3 M NaSCN.

The column may lose activity after prolonged exposure to low pH.

F. Recycling the Column

It is recommended that the column be regenerated immediately after use by washing with three column volumes of glycine-HCl, pH 2.5. The column should be immediately re-equilibrated in PBS until the effluent is at neutral pH. The number of cycles observed will be dependent on variables such as sample condition.

Note: The column may lose activity after prolonged exposure to low pH.

G. Storing the Column

Wash the column with three column volumes of PBS and store the column at 2 °C to 8 °C in PBS containing 15 mM sodium azide.

SN/AC 4/01

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