

Product Specification Sheet

Anti-GST IgG (fusion protein)-Agarose (Aff matrix)

Cat. GST13-AS

Goat Anti-GST IgG-Agarose

SIZE: 0.5 ml

Expression of genes in *E. coli* or yeast or baculovirus offers a convenient system to produce large amounts of recombinant proteins that may otherwise be difficult to isolate from natural cells and tissues. Very often antibodies to these newly identified proteins are not available to study its biochemical properties, monitor protein expression, and purification. In order to circumvent this problem, short pieces of well-defined peptides (Poly-His, Flag-epitope or c-myc epitope or HA-tag) or small proteins (bacterial GST, MBP, Thioredoxin, b-Galactosidase, VSV-Glycoprotein etc) are often cloned along with the target gene. Proteins are expressed as fusion proteins. Antibodies to these fusion-tags are already available to monitor fusion protein expression and purification. Therefore, fusion-tags serve as universal tags much like secondary antibodies. Many tags have their own characteristics. Poly-His-fusion proteins (6 x His) can bind to Nickel-Sepharose or Nickel-HRP. GST-fusion proteins can bind to glutathione-Sepharose. Therefore, a high degree of purification of fusion protein can be achieved in just one affinity purification step. Purity of fusion proteins can be followed by Tag-antibodies. Very often, fusion proteins are directly injected into animals to generate antibodies. Some fusion tags can be removed later by treatment with enzymes to generate tag-free recombinant proteins.

Source of Antigen and Antibodies

Bacterial GST (*Schistosoma japonicum*, ~27 kda) was expressed in *E. coli* and purified (>97%). Purified GST was injected into **goats** to produce high titer polyclonal antibodies. Antibodies (IgG) were purified by delipidation, salt fractionation, and ion-exchange (IgG) (**cat # GST13-A**). It is supplied in PBS, pH 7.4 at 1 mg/ml in liquid or powder form. Dissolve in 100 ul water and use at 1:1K-1: 5K for Western and 1:1K-: 10K for ELISA.

Anti-GST IgG was coupled to agarose at 2-5 mg/ml of beads (**Cat # GST13-AS**). The affinity matrix is supplied in PBS pH 7.4 containing 0.05% azide. The column has a binding capacity of approx. 5 mg/ml of beads.

Store at 4oC. DO NOT FREEZE.

Recommended Usage

Anti-GST IgG-Agarose may be used for immunoprecipitation and for affinity purification of fusion proteins containing the GST tag. The binding capacity of the column for the affinity purification of the fusion protein must also be evaluated for a given fusion protein.

Antibody concentration must be optimized for each application under defined experimental conditions.

Immunoprecipitation

25-50 ul of gel volume (50-10 ul of 1:1 suspension) per 500 ug of the protein lysate. For recombinant proteins with high level of expression, the lysate amounts must be

optimized. It is recommended to use the pre-clared cell lysate. Binding can be performed in RIPA buffer and eluted with SDS-PAGE sample buffer. Eluted proteins visualized with anti-GST antibody (Cat # GST13-A).

Purification of GST-fusion proteins.

The purification can be performed using a small column or the batch process. Purification can be performed at 4oC (for temp sensitive proteins) or room temp.

1. Apply clear cell lysates to the column and recycle 2-3 times. Cell extracts can be prepared in PBS, pH 7.4 or other suitable buffers.
2. Collect the flow through, and wash until the OD280 is <0.020 or achieved a base line.
3. Elute proteins with 0.1M ammonium hydroxide (pH 11-12) into vials containing 30-50 ul of 1N acetic acid per ml of eluant. Collect 0.5-1 ml fraction (5-10 ml total).
4. Low pH elution (Tris-Gly pH 2.5, followed by neutralization in 1M Tris pH 8.0) can also be used.
5. Affinity column should not be exposed to low or high pH for prolonged periods of time.
6. Binding and elution buffers must be optimized for a given protein.
7. Bound proteins should be dialyzed against PBS or other buffer at 4oC and concentrated if necessary.

Specificity

Purified recombinant GST protein (Cat #GST11-R) and Anti-Gst (#GST13-A and #GST12-M etc) are available to optimize the aff matrix purification or IP conditions.

This product is for in vitro research use only.

Related material available from ADI

Purified GST, Monoclonal anti-GST, GST Coated ELISA plates; Anti-Goat HRP conjugates'

Western blot Recycling Kit; Strips antibodies in 5-10 min at room temp.

GST13-AS

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