Anti-GST Antibody

Goat Anti-GST Antibody from goat serum

Product Specification Sheet

Introduction

Product codes

27457750 50 mg

27457762 10 mg

27457701 2.5 mg

Important

Read these instructions carefully before using the products.

Intended use

The products are intended for research use only, and shall not be used in any clinical or *in vitro* procedures for diagnostic purposes.

Safety

For use and handling of the products in a safe way, refer to the Safety Data Sheets.

WARNING
Contains Sodium Azide in dilute solution. Dispose of waste by flushing with copious amounts of water to avoid the build up of explosive metallic azides in copper and lead plumbing.

Storage

Store at 2–8°C.

Components

Anti-GST Antibody

Purified from goat serum and supplied in 150 mM NaCl and 0.02% sodium azide (1:1000-1:2000 working dilution).

Note: *After storage, a small amount of precipitation may be observed in the tube. It should not affect the performance of the antibody.*

The antibody is provided unconjugated to allow maximum flexibility in choice of detection systems. The Anti-GST Antibody can be readily detected using a secondary antibody such as an anti-goat IgG Alkaline Phosphatase conjugate available from Sigma Chemical Company (Product code A-7650).

Quality control

Anti-GST Antibody is tested for its ability to detect, with low background, 100 ng of a GST fusion protein on a Western blot prepared using the Anti-GST Antibody at 1:2000 dilution.

Additional reagents required

Western blot

- SDS-PAGE apparatus with elecroblotting cell [e.g. PhastSystem™ Separation-Control and Development Units 120 VAC (18101823) with PhastTransfer Kit (18100123)].
- Transfer membrane
- 10 x PBS: 1.4 M NaCl, 27 mM KCl, 101 mM Na_2HPO_4, 18 mM KH_2PO_4, pH 7.3.
- Blocking/incubation buffer: 10% w/v non-fat dry milk, 1 x PBS, 0.3% Tween™ 20.
- Wash buffer: 1 x PBS, 0.3% Tween 20.
- Reagents for blot development.

Protocol

Detection of GST fusion by Western blot

Note: *A secondary antibody for the detection of the Anti-GST Antibody is not included and must be purchased from another supplier (e.g. Anti-Goat IgG Alkaline Phosphatase Conjugate; Sigma Product Code A-7650).*

Step	Action
1	Fractionate the protein samples by SDS-PAGE. ***Note:*** *The Anti-GST Antibody has been cross-adsorbed against E. coli proteins. However, this process may not remove all cross-reacting antibodies. We suggest that a sample of an E. coli lysate made from a culture that does not contain a pGEX plasmid be run as a control.*
2	Transfer the separated proteins from the gel to an appropriate membrane (i.e. nitrocellulose, nylon, etc.). ***Note:*** *Electrophoresis and protein transfer may be accomplished using a variety of equipment and reagents. Using PhastSystem (18101823) and PhastTransfer (18100123) from Cytiva, protein blots may be prepared in less than 1 hour.*
3	Proceed with the next part of the protocol.

Blocking of membrane

Step	Action
1	Transfer the membrane onto which the proteins have been blotted into an appropriate container (e.g. petri dish or small baking dish).
2	Add 50–200 ml of blocking/incubation buffer (see *Additional reagents required, on page 1*).
3	Incubate for 1–16 hours at ambient temperature with gentle shaking. ***Note:*** *Longer incubation times (up to 16 hours) with blocking buffer may reduce background signal.*
4	Decant and discard the buffer.
5	Proceed with the next part of the protocol.

Anti-GST Antibody

Step	Action
1	Prepare a 1:1000–1:2000 dilution of Anti-GST Antibody with blocking/incubation buffer (see *Additional reagents required, on page 1* e.g. 10 µl of antibody to 10 ml of buffer). ***Note:*** *The recommended dilution may not be optimal for all assays. If required, a titration of the Anti-GST Antibody should be performed to determine the optimal dilution.*
2	Pour the antibody/buffer mixture into the container with membrane.
3	Incubate for 1 hour at ambient temperature with gentle mixing.
4	Decant and discard the antibody/buffer.
5	Rinse twice with 20–30 ml of wash buffer (see *Additional reagents required, on page 1*) to remove the majority of unbound antibody.
6	Decant and discard the rinses.
7	Wash the membrane with 20–30 ml of wash buffer for 10 minutes at ambient temperature with gentle mixing.
8	Discard the wash buffer and repeat.
9	Proceed with the next part of the protocol.

Secondary antibody

Step	Action
1	Dilute the secondary antibody (e.g. Anti-Goat IgG Alkaline Phosphatase Conjugate; Sigma Product code A-7650) with blocking/incubation buffer (see *Additional reagents required, on page 1*) according to the manufacturer's recommendation.
2	Pour the antibody/buffer mixture into the container with the membrane.
3	Incubate for 1 hour at ambient temperature with gentle mixing.
4	Decant and discard the antibody/buffer.
5	Rinse twice with 20–30 ml of wash buffer (see *Additional reagents required, on page 1*) to remove the majority of unbound antibody.
6	Decant and discard the rinses.
7	Wash the membrane with 20–30 ml of wash buffer for 10 minutes at ambient temperature with gentle mixing.
8	Discard the wash buffer and repeat.
9	Develop the blot with the appropriate substrate for the conjugated secondary antibody.

Related products

PhastSystem	18101823
PhastTransfer	18100123

cytiva.com

For local office contact information, visit cytiva.com/contact

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