

Amersham Anti-GST-HRP Conjugate

Product Specification Sheet

Introduction

Product code

RPN1236

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.

Storage

Store at -15°C to -30°C.

Expiry

The expiry date is printed on the outer packaging.

Components

Anti-GST-HRP conjugate: Horseradish Peroxidase (HRP) conjugated to goat anti-GST polyclonal antibody. 75 µL supplied in Phosphate buffered saline containing 50% Glycerol.

Other materials required

- SDS-PAGE and electroblotting equipment.
- Nitrocellulose transfer membrane: eg RPN2020D
- ECL blocking agent: RPN2125
- Phosphate-buffered saline: (PBS), pH 7.5. Dissolve 11.5 g Di-Sodium Hydrogen Orthophosphate anhydrous (80 mM), 2.96 g Sodium Dihydrogen Orthophosphate (20 mM) and 5.84 g Sodium Chloride (100 mM) in 1000 ml distilled water
- PBS- Tween (PBST): Dilute the required amount of Tween™20 in buffer. A 0.1% Tween 20 concentration is suitable for most blotting applications.
- Reagents for blot development

Description

The anti-GST antibody is supplied conjugated to HRP allowing GST fusion proteins to be detected by a variety of methods. The conjugate can be detected by chemiluminescence using ECL™.

Detection Reagents and ECL Plus Detection Regents available from Cytiva. The conjugate can also be detected using colorimetric methods such as 3,3-Diaminobenzidine (DAB) and 3,3', 5,5'-Tetramethylbenzidine (TMB) available from Sigma-Aldrich.

Detection of GST fusion

Step Action

- 1 Perform SDS-PAGE electrophoresis on the protein samples according to standard techniques.
Note:
The anti-GST antibody has been cross-absorbed against E.coli proteins. However, this process may not remove all crossreacting 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 proteins onto a membrane using standard protocols. Nitrocellulose is recommended for optimum results however, PVDF has been shown to give comparable results.

Immunodetection of membrane

Step Action

- 1 Block non-specific binding sites by immersing the membrane in 5% non-fat dried milk (we recommend ECL blocking agent) in PBST, (see [Other materials required, on page 1](#)). Incubate for 1 hour on a platform shaker at room temperature.
- 2 Briefly rinse the membrane in two changes of PBST wash buffer.
- 3 Dilute the anti-GST/HRP conjugate in PBST. A 1:5000 dilution has been found to be satisfactory for many applications. Allow sufficient antibody solution to cover the membrane (at least 0.25 mL/cm² membrane). Incubate the membrane in diluted conjugate for 1 hour at room temperature on a platform shaker.
- 4 Briefly rinse the membrane in two changes of PBST wash buffer. Wash the membrane in >4 mL/cm² of wash buffer for 15 minutes at room temperature with gentle shaking.
- 5 Wash the membrane for 3 x 5 minutes with fresh changes of wash buffer at room temperature with gentle shaking.
- 6 Develop the blot with the required substrate according to manufacturers instructions.

Related products

ECL Detection Reagents	RPN2109
ECL Blocking Agent	RPN2125
ECL Plus Detection Reagents	RPN2132
GST Western Blotting Detection Kit	RPN1237

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29460019 AA V:3 11/2020

