Advanced Targeting Systems

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HRP-labeled Antibody to Glutathione S-Transferase
RABBIT POLYCLONAL

Catalog Number: AB-45HRP
Quantity: 100 micrograms
Format: PBS (0.14 M Sodium Chloride; 0.003 M Potassium Chloride; 0.002 M Potassium Phosphate; 0.01 M Sodium Phosphate; pH 7.4).
Host: Rabbit
Immunogen: recombinant GST

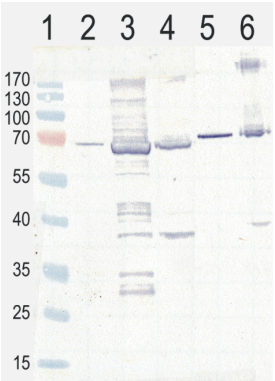
Background: The GST family of enzymes comprises a long list of cytosolic, mitochondrial, and microsomal proteins that are 45-55 kDa (dimer form) size and are capable of multiple reactions with a multitude of substrates, both endogenous and xenobiotic. GST catalyzes the conjugation of reduced glutathione, meaning the sulfhydryl group, to electrophilic centers on a wide variety of substrates. This activity is useful in the detoxification of endogenous compounds such as peroxidized lipids, as well as the metabolism of xenobiotics. GST binds toxins and functions as a transport protein. Glutathione S-transferase is used to create the GST gene fusion system in which the GST moiety is used to detect and purify proteins of interest. In a GST gene fusion system, the GST sequence is incorporated into an expression vector in frame with the gene sequence encoding the protein of interest. Induction of protein expression results in production of a fusion protein - the protein of interest fused to GST. This GST-fusion protein can then be purified from cell extracts via its high affinity for glutathione. Fusion proteins offer an important biological assay for direct protein-to-protein interactions. The GST tag is a 220 amino acid protein usually fused to the N-terminus of a protein; compared to other tags like myc or FLAG it is quite big. However, many commercially-available sources of GST-tagged plasmids include a thrombin domain for cleavage of the GST tag during protein purification. GST-fusion proteins can be produced in *Escherichia coli* as recombinant proteins. The HRP-labeled GST antibody is used for the detection of GST fusion proteins.

Specificity & Preparation: This antibody recognizes glutathione S-transferase (GST). Recombinant GST was used as the immunogen. The antibody is conjugated to horseradish peroxidase (HRP) and affinity purified over a GST-agarose column.

Usage: Applications include immunoblotting (ATS in-house: western blot 1:500-1:1000).

Storage: Store the antibody at 4°C for short periods only (less than 24 hours) or -20°C in undiluted aliquots for up to one year. Avoid repeated freezing and thawing. Precautions should be taken to avoid rapid thawing cycles by adding 40-50% glycerol. Gently spin down material before use; 5-10 seconds in a microfuge should be adequate.

To view protocol(s) for this and other products please visit: www.ATSbio.com/support/protocols



Cultures of *E. coli* transformed with a vector expressing a GST-Cre recombinase fusion protein were grown under varying conditions. Soluble protein was extracted from two different clones and expression compared between soluble and insoluble fractions on SDS-PAGE which was then transferred to a PVDF membrane. Membrane was incubated with anti-GST-HRP (Cat. #AB-45HRP) overnight at a 1:500 dilution.

Lane 1: Page Ruler 5 μl

Lane 2: 200 ng GST-Cre

Lane 3: Supernate 20 μg
Lane 4: Supernate 20 μg

Lane 5: Pellet 5 μl