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Product Information

Anti-VSV-G–Peroxidase antibody, Mouse monoclonal

clone P5D4, purified from hybridoma cell culture

A5977

Product Description

Monoclonal Anti-VSV-G–Peroxidase is a lyophilized preparation of the purified immunoglobulin fraction of Monoclonal Anti-VSV-G (mouse IgG1 isotype) isolated from ascites fluid of the P5D4 hybridoma, conjugated to horseradish peroxidase (HRP). The antibody is derived from the P5D4 hybridoma produced by the fusion of mouse myeloma cells and splenocytes from BALB/c mouse immunized with a synthetic peptide containing the 15 carboxy-terminal amino acids (497-511) of Vesicular Stomatitis Virus Glycoprotein (VSV-G), conjugated to KLH.

Anti-VSV-G–Peroxidase recognizes the VSV-G tag sequence (YTDIEMNRLGK) on VSV-G tagged fusion proteins when expressed N- or C-terminal to the fusion protein. The antibody reacts specifically with VSV-G tagged fusion proteins by immunoblotting, and the reaction is specifically inhibited by the VSV-G Tag peptide (Cat No. V7887).

Epitope tags provide a method to localize gene products in a variety of cell types, to study the topology of proteins and protein complexes, and to identify associated proteins. In addition, it allows characterization of newly identified, low abundance or poorly immunogenic proteins when protein specific antibodies are not available.[1-3] Utilizing a viral epitope as a tag minimizes the risk of having the same epitope in cellular proteins, and thus, the possibility of antibody cross-reaction with cellular material.

The amino acid sequence YTDIEMNRLGK, corresponding to amino acids 501-511 of the Vesicular Stomatitis Virus glycoprotein (VSV-G), has been widely used as an epitope tag in expression vectors, enabling the expression of proteins as VSV-G tagged fusion proteins.[1-4]

The Vesicular Stomatitis Virus Glycoprotein (VSV-G) constitutes an attractive model to study maturation and intracellular transport of membrane proteins.[5] It mediates attachment of VSV to the cell surface and induces pH-dependent fusion between viral and target membranes.[4] In addition, its cytoplasmic domain contains information for several intracellular sorting steps, which include efficient export from the ER, basolateral delivery and endocytosis.[6] Transport between Golgi cisternae was shown to be unidirectional by assaying "donor" populations of Golgi membranes containing VSV-G, and "acceptor" populations, containing an enzyme that adds to it N-acetylglucosamine.[7] Temperature sensitive mutants of VSV-G are used to study exit of folding intermediates from the endoplasmic reticulum.[8]

Reagent

Supplied as a lyophilized powder. After reconstitution, the solution contains 1% BSA and 0.05% MIT in 0.01 M sodium phosphate buffered saline, pH 7.4.

Antibody Concentration

1.0-1.5 mg/mL.

Molar ratio Ab/Enzyme

0.6 to 1.4

Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

Reconstitute the vial contents with 0.5 mL distilled water.

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Storage/Stability

Stored the lyophilized product at 2-8 °C.

After reconstitution, for extended storage, freeze in working aliquots at –20 °C. For continuous use, the solution may be stored at 2-8 °C for up to 1 month. Working dilutions should be discarded. Avoid repeated freeze-thaw.

Product Profile

Immunoblotting

A working antibody dilution of 1:1,000 detects 20-50 ng of a purified VSV-G tagged fusion protein.

Note: In order to obtain best results and assay sensitivity in different techniques and preparations we recommend determining optimal working dilutions by titration.

Procedure

All incubation steps should be performed at room temperature.

1. Separate VSV-G tagged proteins from sample lysates using a standard SDS-PAGE protocol. Load 2.5-20 μg total lysate protein per lane. The amount of lysate to be loaded depends on the level of protein expression and may vary between experiments.
2. Transfer proteins from the gel to a nitrocellulose membrane.
3. Block the membrane using a solution of 5% non-fat dry milk in PBS for 60 minutes. PBS, Cat. No. D8537; non-fat dried milk, Cat. No. M7409
4. Wash the membrane three times for 5 minutes each in PBS containing 0.05% TWEEN® 20, Cat. No. P3563.
5. Incubate the membrane with Monoclonal Anti-VSV-G-Peroxidase diluted in PBS containing 0.05% TWEEN® 20 for 120 minutes.
6. Wash the membrane three times for 10 minutes each in PBS containing 0.05% TWEEN® 20.
7. Treat the membrane with a peroxidase substrate.

References

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