

Product Information

Anti-VSV-G

produced in rabbit, affinity isolated antibody

Catalog Number **V4888**

Product Description

Anti-VSV-G is produced in rabbit using a synthetic peptide corresponding to amino acids 497-511 of the Vesicular Stomatitis Virus glycoprotein (VSV-G) conjugated to maleimide activated KLH via a cysteine residue added at the carboxyl terminus. The antibody is affinity-purified using the immunizing peptide immobilized on agarose.

Anti-VSV-G reacts specifically with amino- and carboxyl-terminal VSV-G tagged fusion proteins by immunoblotting, immunoprecipitation, and immunocytochemistry (immunofluorescence staining of transfected cells). Reaction of the antibody in immunoblotting is specifically inhibited by the VSV-G tag peptide, Catalog Number 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, the tag allows characterization of newly identified, low abundance or poorly immunogenic proteins when protein-specific antibodies are not available.¹⁻³ Engineering a viral epitope as a tag minimizes the risk of having the same epitope in cellular proteins, and thus, minimizes the possibility of antibody cross-reaction with cellular material. The epitope is located within amino acids 501-511 of the Vesicular Stomatitis Virus glycoprotein (VSV-G) containing the sequence YTDIEMNRLGK. It has been widely used as a tag in expression vectors, enabling the expression of proteins as VSV-G tagged fusion proteins.^{1,4} Anti-VSV-G recognizes this epitope, and can be used to identify the successful expression of the tagged protein. This epitope is identical to the epitope recognized by the P5D4 monoclonal antibody, Catalog Number V5507.

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, including efficient export from the endoplasmic reticulum, 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 N-acetylglucosamine to VSV-G.⁷ Temperature-sensitive mutants of VSV-G have been used to study the exit of protein-folding intermediates from the endoplasmic reticulum.⁸

Reagent

Solution in 0.01 M phosphate buffered saline, pH 7.4, containing 1% bovine serum albumin and 15 mM sodium azide as a preservative.

Antibody concentration: ~1.0 mg/ml.

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

Store at -20 °C. For continuous use, the product may be stored at 2-8 °C for up to one month. For extended storage, freeze in working aliquots at -20 °C. Repeated freezing and thawing, or storage in a frost-free freezer, is not recommended. If slight turbidity occurs upon prolonged storage, clarify the solution by centrifugation before use. Working dilutions should be discarded if not used within 12 hours.

Product Profile

Immunoblotting: a minimum of 0.1-0.2 µg/ml of the antibody detects VSV-G tagged fusion proteins from transfected mammalian cell lysates.

Indirect immunofluorescence staining: a minimum of 1.0 µg/ml of the antibody detects VSV-G tagged fusion proteins in methanol/acetone fixed transiently transfected cells.

Immunoprecipitation: a minimum of 0.5 µg of the antibody can immunoprecipitate VSV-G-tagged fusion proteins from transfected mammalian cell lysates.

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

Procedures

Immunoblotting

All incubation steps should be performed at room temperature.

1. Separate VSV-G-tagged proteins from the 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, thus, the optimum loading 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 phosphate buffered saline (PBS, Catalog Number D8537) for 60 minutes.
4. Wash the membrane three times for 5 minutes each in PBS containing 0.05% TWEEN® 20, Catalog Number P3563.
5. Incubate the membrane with Anti-VSV-G as the primary antibody in PBS containing 0.05 % TWEEN 20 and 1% bovine serum albumin (BSA, Catalog Number A9647) for 120 minutes.
6. Wash the membrane three times for 15 minutes each in PBS containing 0.05% TWEEN 20.
7. Incubate the membrane with Anti-Rabbit IgG-Peroxidase (Catalog Number A0545) or Anti-Rabbit IgG- Alkaline-Phosphatase (Catalog Number A9919) as the secondary antibody at the recommended concentration in PBS containing 0.05% TWEEN 20. Incubate for 60 minutes. Adjust the antibody concentration to maximize detection sensitivity and to minimize background.
8. Wash the membrane three times for 5-15 minutes each in PBS containing 0.05% TWEEN 20.

9. Treat the membrane with a Peroxidase or Alkaline Phosphatase substrate.

Indirect Immunofluorescent Staining of Cultured Cells

All incubation steps, except steps 1 and 3, should be performed at room temperature.

1. Grow transfected cultured cells expressing the VSV-G-fusion protein of choice on sterile coverslips at 37 °C.
2. Wash the cells briefly in PBS (Catalog No. D8537).
3. Fix the cells with -20 °C methanol (10 minutes) and then with -20 °C acetone (1 minute); OR fix with 3-4% paraformaldehyde (10 minutes), rinse briefly with PBS, then permeabilize with 0.5% Triton® X-100 (2 minutes).
4. Wash the fixed cells twice in PBS (5 minutes each wash).
5. Incubate the coverslips cell-side-up with Anti-VSV-G as primary antibody in PBS containing 1% BSA. Incubate at room temperature for 1 hour.
6. Wash three times in PBS (5 minutes each wash).
7. Incubate the coverslips cell-side-up with Anti-Rabbit IgG-FITC, (Catalog No. F9887) as the secondary antibody at the recommended dilution in PBS containing 1% BSA. Incubate at room temperature for 30 minutes.
8. Wash three times in PBS (5 minutes each wash).
9. Add one drop of aqueous mounting medium on the coverslip and invert carefully on a glass slide. Avoid air bubbles.
10. Examine using a fluorescence microscope with appropriate filters

Note: Blocking with PBS containing 1% BSA for 10 minutes at room temperature followed by draining prior to step 5 may minimize non-specific adsorption of the antibodies.

Immunoprecipitation

Note: the amount of cell extract to be used for immunoprecipitation depends on the level of expression of the tagged protein and the specific application.

1. Centrifuge 40 µL of Protein A-Agarose Fast Flow, 1:1 suspension (Catalog No. P3476), for 1 minute at 12,000 ×g, and then wash twice with 1 mL RIPA buffer (50 mM Tris base, 0.25% (w/v) deoxycholate, 1% IGEPAL® CA-630, 150 mM NaCl, 1mM EDTA, pH 7.4) at 4 °C.
2. Add Anti VSV-G antibody diluted in PBS, and incubate with continuous inversion for 1 hour.

3. Centrifuge for 1 min 12,000 $\times g$, wash twice with 1 mL RIPA at 4 °C.
4. Add 0.1-1.0 mL of cell extract containing VSV-G-tagged protein to the beads (see Note above), and incubate from 2 hours to overnight at 4 °C with continuous inversion.
5. Centrifuge the beads and remove the supernatant.
6. Wash the beads five times with 1 ml RIPA each wash, by vortex mixing the beads followed by a brief centrifugation.
7. Resuspend the pellet in 25 μ L 2 $\times$ SDS-PAGE sample buffer. Boil the sample for 5 minutes and centrifuge. The sample is ready to be loaded on an SDS-PAGE gel.

References

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