3050 Spruce Street
Saint Louis, Missouri 63103 USA
Telephone (800) 325-5832 (314) 771-5765
Fax (314) 286-7828
email: techserv@sial.com
sigma-aldrich.com

ProductInformation

MONOCLONAL ANTI- VSV GLYCOPROTEIN
CLONE P5D4
Mouse Ascites Fluid

Product Number **V5507**

Product Description

Monoclonal Anti-VSV Glycoprotein (mouse IgG1 isotype) is derived from the P5D4 hybridoma produced by the fusion of mouse myeloma cells and splenocytes from a 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.[1] The isotype is determined using Sigma ImmunoType™ Kit (Product Code ISO-1) and by a double diffusion immunoassay using Mouse Monoclonal Antibody Isotyping Reagents (Product Code ISO-2).

Monoclonal anti-VSV Glycoprotein (VSV-G) recognizes an epitope containing the five carboxy-terminal amino acids of VSV Glycoprotein.[1,2] In infected cells, the antibody localizes the immature forms of VSV-G in the rough endoplasmic reticulum (RER) and in the cisternae of Golgi complex, as well as mature VSV-G at the cell surface and in the budding virus, but not the secreted form of VSV-G, lacking the membrane and the cytoplasmic domain.[1] This antibody has been used in studies applying microinjection of antibody,[1,2] immunoblotting,[1,3] immunoprecipitation,[4-7] immunocytochemistry[1,4-6,8-10] and immunoelectron microscopy.[1,2,5,10] The antibody has been used for the detection, immunoprecipitation and immunocytochemical staining of fusion proteins tagged with the sequence recognized by the P5D4 antibody, which is known as VSV-G tag.[4]

Recombinant DNA technology enables the attachment of genes of interest to specific sequences or genes that can provide 'affinity handles' (tags) designed to enable the selective identification and purification of the protein of interest.[11-13] These sequences of tails or tags are genetically engineered away from the protein active site, by insertion at the N- or C-terminus. Engineering 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 envelope of vesicular stomatitis virus (VSV) consists of a bilayer membrane with a single type of glycoprotein, the G-protein (VSV-G) which mediates attachment to the cell surface and induces pH-dependent fusion between viral and target membranes.[14] The carboxyl terminus of the VSV-G protein which does not have any homology with cellular proteins, has been engineered into expression vectors as a tag. Proteins expressed with this tag may thus be detected and localized using an antibody reactive specifically against this epitope with no risk of cellular background staining.[2,4]

VSV-G has also become an attractive model to study maturation and intracellular transport of membrane proteins. Antibodies that react specifically against VSV-G have been used for studies on the role of the cytoplasmic domain of newly-synthesized VSV-G during transfer to the plasma membrane and cell surface. Thus for instance, microinjection of specific antibodies has proven to be a powerful approach to study the function of cytoplasmic proteins *in vivo*. Such antibodies are also useful for *in vitro* studies on virus-host cell interactions applying immunoblotting, immunoprecipitation, immunocytochemistry and electron microscopy.[1,2]

Reagent

Monoclonal anti-VSV Glycoprotein is supplied as ascites fluid with 15 mM sodium azide.

Precautions and Disclaimer

Due to the sodium azide content a material safety data sheet (MSDS) for this product has been sent to the attention of the safety officer of your institution. Consult the MSDS for information regarding hazardous and safe handling practices.

Storage/Stability
For continuous use, store at 2 °C to 8 °C for up to one month. For extended storage, freeze in working aliquots. Repeated freezing and thawing is not recommended. Storage in "frost-free" freezers is not recommended. If slight turbidity occurs upon prolonged storage, clarify the solution by centrifugation before use. Solutions at working dilution should be discarded if not used within 12 hours.

Product Profile
A minimum working dilution of 1:100,000 is determined by immunoblotting, using a whole cell extract of mammalian or bacterial cells expressing a protein tagged with the carboxyl-terminus of VSV glycoprotein or a whole extract of VSV virus.
Note: In order to obtain best results in different techniques and preparations we recommend determining optimal working dilution by titration test.

Procedure
Immunoblotting
All incubation steps should be performed at room temperature.
1. Separate VSV-G-tagged proteins from sample lysates using a standard sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) protocol. Load 2.5 to 20 µg of total lysate protein per lane. The amount of lysate to be loaded per lane 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 phosphate buffered saline (PBS, Product No. D8537) for at least 60 minutes.
4. Wash the membrane three times for 5 minutes each in PBS containing 0.05 % Tween 20 (Product No. P3563).
5. Incubate the membrane with Anti-VSV-G antibody as the primary antibody using an optimized concentration in PBS containing 1 % bovine serum albumin (BSA, Product No. A9647) for two hours.
6. Wash the membrane three times for 5 minutes each in PBS containing 0.05 % Tween 20.
7. Incubate the membrane with Anti-mouse IgG Peroxidase conjugate (e.g. Product No. A9917, A3682, or A2304) or with Anti-mouse Alkaline Phosphatase conjugate (e.g. Product No. A1293, A2179 or A1682) 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 minutes each in PBS containing 0.05 % Tween 20.
9. Treat the membrane with either a peroxidase or an alkaline-phosphatase substrate as appropriate.

References

1. Kreis, T.E., EMBO J., **5**, 931-941 (1986).
2. Kreis, T.E., and Lodish, H.F., Cell, **46**, 929-937 (1986).
3. Gautreau, A., et al., Proc. Natl. Acad. Sci. USA, **96**, 7300-7305 (1999).
4. Soldati, T., and Perriard, J.-C., Cell, **66**, 277-289 (1991).
5. Hammond, C., and Helenius, A., J. Cell Biol., **126**, 41-52 (1994)
6. Li, J., et al., Proc. Natl. Acad. Sci. USA, **94**, 502-507 (1997).
7. Prenzel, N., et al., Nature, **402**, 884-888 (1999).
8. Duden, R., et al., Cell, **64**, 649-665 (1991).
9. Hamm-Alvarez, S.F., et al., Proc. Natl. Acad. Sci. USA, **91**, 7812-7816 (1994).
10. Griffiths, G., et al., J. Cell Sci., **108**, 2839-2856 (1995).
11. Narayanan, S.R., J. Chromatogr., **658**, 237-258 (1994).
12. Casey, J.L., et al., J. Immunol. Meth., **179**, 105-116 (1995).
13. Uhlen, M., and Moks, T., Meth. Enzymol., **185**, 129-143 (1990).
14. Ohnishi, S., Curr. Top. Membr. Transp., **32**, 257-296 (1988).

SN/AC 2/01