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
Cy3 CONJUGATE
CLONE P5D4

Product Number **C 7706**

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). The immunoglobulin fraction of antibody to VSV-G is purified from ascites fluid produced from the P5D4 hybridoma using Protein A and then conjugated to Cy3. The conjugate is then extensively dialyzed to remove unbound Cy3.

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] The unconjugated antibody has been used in studies applying microinjection of antibody,[1,2] immunoblotting,[1,3] immunoprecipitation,[4-7] immunocyto- chemistry[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] The Cy3 conjugated antibody has been used for immunofluorescent staining of cultured cells expressing VSV-G tagged fusion protein.

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 for studying 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, immuno-cytochemistry and electron microscopy.[1,2]

Reagent
Monoclonal Anti-VSV Glycoprotein is provided as a solution in 0.01 M phosphate buffered saline pH 7.4 containing 1% BSA and 15 mM sodium azide.

Specific Antibody Concentration: 0.5 to 2 mg/ml
Molar ratio (F/P) of the product: 3.0 to 9.0
Spectral Characteristics of Cy3:[15]
Absorbance Max = 552 nm
Emission Max = 570 nm

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 and extended storage, store at 2 °C to 8 °C. Protect from prolonged exposure to light. 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 1:10,000 is determined by direct immunofluorescent staining of transfected COS-7 cells expressing 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 test.

Procedure
Direct Immunofluorescent Staining of Cultured Cells
All incubation steps should be performed at room temperature.

1. Grow transfected cultured cells expressing VSV-G tagged fusion protein of choice on sterile coverslips at 37 °C.
2. Wash the cells briefly in phosphate buffered saline (PBS, Product No. D 8537).
3. Fix the cells with 0.37 % paraformaldehyde containing 0.05 % Triton X-100 (5 minutes) and then with 3.7 % paraformaldehyde (15 minutes).
4. Wash coverslips twice in PBS (5 minutes each wash).
5. Incubate coverslips cell-side-up with anti-VSV-G, Cy3 conjugate in PBS containing 1 % BSA (BSA, Product No. A 9647). Incubate for 60 minutes.
6. Wash three times in PBS (5 minutes each wash).
7. Add one drop of aqueous mounting medium on the coverslip and invert carefully on a glass slide. Avoid air bubbles.
8. Examine using a fluorescence microscope with appropriate filters.

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**, 57-296 (1988).
15. Southwick, P.L., et al., Cytometry, **11,** 418-430 (1990).

SN/AC 2/26/01