**MONOCLONAL ANTI-VSV GLYCOPROTEIN**
**CLONE P5D4**
**Cy3 Conjugate**
**Purified Mouse Immunoglobulin**

Product No. **C7706**
Lot 085H4851

Monoclonal Anti-VSV Glycoprotein (mouse IgG1 isotype) is derived from the P5D4 hybridoma produced by the fusion of mouse myeloma cells and splenocytes from immunized BALB/c mice. A synthetic peptide containing the 15 carboxy-terminal amino acids (497-511) of Vesicular Stomatitis Virus Glycoprotein (VSV-G), conjugated to KLH was used as immunogen. The isotype is determined using Sigma ImmunoType™ Kit (Sigma ISO-1) and by a double diffusion immunoassay using Mouse Monoclonal Antibody Isotyping Reagents (Sigma ISO-2). The product is prepared by conjugation of Cy3[1] to Protein A purified Monoclonal Anti-VSV Glycoprotein. The Cy3-antibody conjugate is then extensively dialyzed to remove unbound Cy3 fluorophore. The product is provided as a solution in 0.01 M phosphate buffered saline, pH 7.4, containing 1% BSA and 0.1% sodium azide (see MSDS)* as a preservative.

**Specificity**
Monoclonal Anti-VSV Glycoprotein (VSV-G) recognizes an epitope containing the five carboxy-terminal amino acids of VSV Glycoprotein.[2] In infected cells, the antibody localizes the immature forms of VSV-G in the rough endoplasmic reticulum (RER) and in the cisternae of the 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 which lacks the membrane and the cytoplasmic domain. This antibody has been used for studies on the role of the cytoplasmic domain on newly-synthesized VSV-G during transfer to the plasma membrane and cell surface, applying microinjected antibody, immunoblotting, immunoprecipitation, immunocytochemistry and immunoelectron microscopy.[2,3] The antibody has been used for the detection, immunoprecipitation and immunocytochemical staining of exogenously introduced constructs tagged with carboxyl-terminus of VSV-G.[4] This tag does not interfere with the function of the studied protein and can be specifically recognized by the P5D4 antibody without cross-reaction with any endogenous protein.

**Antibody Content:** 1.0 mg/ml by absorbance at 280 nm.

**Spectral Characteristics of Cy3**

| | |
|---|---|
| Absorbance Max | 552 nm |
| Emission Max | 570 nm |

**F/P Molar Ratio:** 6.7 (prior to addition of BSA)
The F/P molar ratio of the Cy3-antibody conjugate is determined spectrophotometrically as follows:

$$F = A_{552}/0.14 \quad P = \frac{A_{280} - (A_{552} \times 0.05)}{1.4}$$

F/P Molar Ratio = F/P x 0.16
Where:

| | | |
|---|---|---|
| 0.14 | = | extinction coefficient of Cy3 at $A_{552}$. |
| 1.4 | = | extinction coefficient of IgG at $A_{280}$. |
| 0.05 | = | correction factor for Cy3 absorbance at $A_{280}$. |
| 0.16 | = | correction factor for molecular weights of Cy3 and IgG |

**Working Dilution**
A dilution of 1:10,000 was determined by direct immunofluorescence using COS-7 cultured cells transfected with a VSV-G peptide-tagged fusion protein contruct.

In order to obtain best results, it is recommended that each individual user determine the optimum working dilution for their system by titration assay.

**Description**
The introduction of a "tag" into the sequence of a protein of interest enables the expression of the cloned protein in a wide variety of cells and its detection using a specific antibody, without the difficulty of discriminating it from the endogenous proteins. Choosing a viral epitope as the "tag" minimizes the risk of 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.[5] Using the carboxyl terminus of the VSV-G

protein, it is possible to insert this epitope homotopologically at the C-terminus of cellular proteins to be tagged. Since this small protein stretch does not appear to share epitopes with any protein expressed in uninfected cells, it may be detected and localized by the use of an antibody specifically reactive against an epitope consisting of this protein sequence.[3] 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.

**Uses**

Cy3 Monoclonal Anti-VSV Glycoprotein may be used for:

1. The detection and localization of constructs tagged with carboxyl-terminus of VSV-G.
2. *In vitro* studies on virus-host cell interactions.
3. Double labeling experiments with other fluorescently-tagged antibodies.

**Storage**

Store at 2-8°C. Protect from prolonged exposure to light. If slight turbidity occurs upon prolonged storage, clarify the solution by centrifugation before use.

* Due to the sodium azide content a material safety 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.

**References**


1. Southwick, P., et al., Cytometry, **11**, 418 (1990).
2. Kreis, T., EMBO J., **5**, 931 (1986).
3. Duden, R., et al., Cell, **64**, 649 (1991).
4. Soldati, T., and Perriard, J., Cell, **66**, 277 (1991).
5. Ohnishi, S., Curr. Top. Membr. Transp., **32**, 257 (1988).




12/98