

MONOCLONAL ANTI-VIMENTIN Cy3 CONJUGATE CLONE V9 Purified Mouse Immunoglobulin

Product No. C 9080

Monoclonal Anti-Vimentin (mouse IgG1 isotype) is derived from the hybridoma produced by the fusion of mouse myeloma cells and splenocytes from immunized BALB/c mice. Vimentin, purified from pig eye lens, was used as the immunogen.¹ The isotype is determined using Sigma ImmunoTypeTM 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 Cy3² to Protein A purified Monoclonal Anti-Vimentin, clone V9. 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 15 mM sodium azide (see MSDS)* as preservative.

Specificity

Cy3 Monoclonal Anti-Vimentin specifically reacts with vimentin in mesenchymally derived cell types. The antibody cross reacts with vimentin-expressing cells in human, monkey, bovine, horse, pig, dog, rabbit, rat, hamster, gerbil and rat kangaroo. It can be used for staining of alcohol- or formalin-fixed, paraffin-embedded tissue sections, acetone-fixed frozen sections and cultured cells. The epitope recognized by the antibody is situated between the single cysteine residue and the C-terminus. The epitope is partially sensitive to prolonged formalin fixation and paraffin embedding.

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

Spectral Characteristics of Cy3

Absorbance Max	552nm
Emission Max	570 nm

F/P Molar Ratio 3 to 9

The F/P molar ratio of the Cy3-antibody conjugate is determined spectrophotometrically as follows:

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

F/P Molar Ratio = F/P x 0.16

Where:

- $0.14 = \text{extinction coefficient of Cy3 at } A_{552}$.
- 1.4 = extinction coefficient of IgG at A_{280} .
- 0.05 = correction factor for Cy3 absorbance at

0.16 = correction factor for molecular weights of Cy3 and IgG

Working Dilutions

- 1. A minimum dilution of 1:50 was determined by direct immunofluorescence using formalin-fixed, paraffinembedded human tonsil sections.
- 2. A minimum dilution of 1:200 was determined by direct immunofluorescence using cultured human fibroblasts.

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

Cy3 Monoclonal Anti-Vimentin recognizes vimentin, one of the five major groups of intermediate filament proteins, having a molecular weight of 53 kD. The antibody reacts with normal and pathological tissue of mesenchymally-derived cells, lens tissue and various cultured cells. It localizes vimentin in fibroblasts, lipocytes, endothelial cells, some lymphoid cells, melanocytes, macrophages, and chondrocytes, Vimentin is expressed in breast myoepithelial cells, osteocytes, Langerhans cells of the skin, Schwann cells and astrocytes. The antibody stains tumors derived from these cells, including sarcomas, most lymphomas, melanomas and their metastatic lesions. Co-expression of vimentin with keratin was described in most carcinomas of certain sites (e.g., kidney and thyroid) and certain carcinomas of other sites (e.g., breast). Such co-expression is also found in certain non-neoplastic cells, e.g. epithelial cells, ovarian surface epithelium, rete ovarii, granulosa cells, damaged and regenerating tubular epithelia of the kidney and in cultured epithelial cells.

ProductInformation

Uses

Cy3 Monoclonal Anti-Vimentin may be used for:

- 1. Studies on the expression of vimentin in cultured cells.
- 2. Immunohistochemical and immunocytochemical localization of vimentin in normal and pathological tissue of mesenchymal origin.
- 3. Double labeling experiments with fluoresceintagged antibodies
- 4. Testing the adequacy of tissue fixation and for monitoring the recovery from overfixation achieved by antigen retrieval methodologies.

5.

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.

Selected References

- 1. Osborn, M., et al., Eur. J. Cell. Biol., 34, 137 (1984).
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- 3. Lazarides, E., Nature, 283, 249 (1980).
- 4. Franke, W., et al., Exp. Cell. Res., 123, 25 (1979).
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- Osborn, M., and Weber, K., Laboratory Investigation, 48, 372 (1983).
- 7. Toelle, H., et al., Eur. J. Cell. Biol., 38, 234 (1985).
- 8. Bohn, W., et al., Exp. Cell. Res., **201**, 1 (1992).

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