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ProductInformation

MONOCLONAL ANTI-HUMAN CD57 (HNK1)
CLONE VC1.1
FITC CONJUGATE
Purified Mouse Immunoglobulin

Product No. **F6148**

Product Description

Monoclonal Anti-Human CD57/HNK1 (mouse IgM isotype) is derived from the hybridoma produced by the fusion of mouse myeloma cells and splenocytes from BALB/c mice immunized with a homogenate of cat cerebral cortical area.[1,2] The isotype is determined using the Sigma ImmunoType™ Kit (Product Code ISO-1) and by a double diffusion immunoassay using Mouse Monoclonal Antibody Isotyping Reagents (Product Code ISO-2). The product is prepared by conjugation of fluorescein isothiocyanate (FITC) Isomer I to purified CD57/HNK1 monoclonal antibody. The conjugate is then purified by gel filtration to remove unbound FITC, no free FITC is detectable.

Monoclonal Anti-Human CD57/HNK1 recognizes the CD57/HNK1 (110 kDa) human myeloid cell associated surface glycoprotein. CD57/HNK1 is expressed on a subpopulation of (15-20%) peripheral blood mononuclear cells. Expression of the antigen was described for about 60% of NK active cells and for a T cell subset. It is also found on some B cell lines and B-CLL melanoma lines. The antigen is detected in lymphoid tissues, in certain neuronal subsets, in peripheral nerve Schwann cells and in normal, benign and malignant prostate. neurofibromas, schwannomas, traumatic neuromas and small cell lung carcinomas frequently express HNK1.[1-6] The CD57/ HNK1 antigen appears to be expressed in many species ranging from insects to humans. It is thought to be involved in cellular adhesion events and is expressed on several adhesion-related proteins including most neural immunoglobulin superfamily molecules.[7] The epitope recognized by monoclonal VC1.1 is an N-linked carbohydrate which is present in a variety of glycoproteins and proteoglycans and in some glycolipids.[2-5] It is resistant to formalin fixation and paraffin embedding. The VC1.1 antibody and the HNK1 (Leu7)[8] antibody inhibit the binding of each other, thus suggesting that the epitopes recognized by them are either identical or sufficiently close to cause steric hindrance in a binding assay. Both antibodiesare reactive with the same set of polypeptides (including N-CAM and MAG) in western blotting. Inaddition, both exhibit identical immunohistochemical staining patterns in neuronal subsets in the cerebral cortex, in retina, in peripheral nerve Schwann cells, in lymphoid tissues and in normal, benign and malignant prostate.

F/P Molar Ratio: 3.0 to 8.0

Reagents

The conjugate is provided as purified immunoglobulin (25 µg/ml) in 0.01 M phosphate buffered saline, pH 7.4, containing 1% BSA and 0.1% sodium azide as a preservative.

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.

Performance

When assayed by flow cytometric analysis using 10 µl of the antibody to stain 1 X 10^6, cells a fluorescence intensity and percent population positive is observed similar to that obtained with saturating monoclonal antibody levels.

Uses

FITC Monoclonal Anti-Human CD57/HNK1 may be used for:

1. Identification, quantification and monitoring of NK cells and T cell subsets in peripheral blood, biological fluids, lymphoid organs and other tissues.
2. Studies of neural-tissue glycoproteins and glycolipids.
3. Immunohistological and immunocytochemical identification of normal and malignant prostatic cells, normal and neoplastic schwann cells and small cell lung carcinoma cells, (in frozen sections and in formalin-fixed, paraffin-embedded tissue sections).

In order to obtain best results in different preparations and techniques, it is recommended that each individual user determine their optimum working dilutions by titration assay.

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.

Procedure for Direct Immunofluorescent Staining

Reagents and Materials Needed but Not Supplied

1. a. Whole human blood collected by standard clinical blood evacuation tubes with EDTA, ACD-A or heparin anticoagulant **or**
   b. Human cell suspension (e.g., peripheral blood mononuclear cells isolated on HISTOPAQUE™ (Product Code 1077-1)).
2. Diluent: 0.01M phosphate buffered saline (PBS), pH 7.4, containing 1% BSA and 0.1% NaN_3.
3. FITC conjugated, isotype-matched, nonspecific mouse immunoglobulin (negative control, Product No. F7022).
4. 12 x 75 mm test tubes.
5. Adjustable micropipet.
6. Centrifuge.
7. Counting chamber.
8. Trypan blue (Product No. T0776), 0.2% in 0.01 M PBS, pH 7.4.
9. 2% paraformaldehyde in PBS.
10. Whole blood lysing solution.
11. Flow cytometer.

Procedure

1. a. Use 100 µl of whole blood **or**
   b. Adjust cell suspension to 1 x 10^7 cells/ml in diluent. Cells should be >90% viable as determined by dye exclusion (e.g., trypan blue). For each sample, add 100 µl or 1 x 10^6 cells per tube.
2. Add 10 µl of conjugate to tube(s) containing cells to be stained. Vortex tube gently. Incubate the cells at room temperature (18 – 22 °C) for 30 minutes. Proper controls to be included for each sample are:
   a. An autofluorescence control: 10 µl diluent in place of monoclonal antibody, followed by steps 3 - 7.
   b. A negative staining control: 10 µl of FITC conjugated, isotype-matched non-specific mouse immunoglobulin (Product No. F7022) at the same concentration as test antibody followed by steps 3 - 7.
3. a. If whole blood is used, use lysing solution after incubation and wash cells according to manufacturer's instructions.
   b. If a mononuclear cell suspension is used, proceed to Step. 4.
4. Add 2 ml of diluent to all tubes.
5. Pellet cells by centrifugation at 500 x G for 10 minutes.
6. Remove supernatant by careful aspiration.
7. Resuspend cells in 0.5 ml of 2% paraformaldehyde. Analyze in a flow cytometer according to manufacturer's instructions.

Quality Control

It is advisable to run the appropriate negative controls. Negative controls establish background fluorescence and non-specific binding of the primary and secondary antibodies. The ideal negative control reagent is a mouse monoclonal or myeloma protein which has no reactivity with human cells. It should be isotype-matched to the antibody and of the same concentration and F/P molar ratio as the antibody. The degree of autofluorescence or negative control reagent fluorescence will vary with the type of cells under study and the sensitivity of the instrument used.

References

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