Data Sheet

Anti-HNK-1/N-CAM (CD57) Antibody, Mouse Monoclonal

Clone VC1.1, purified from hybridoma cell culture

C6680

Product Description

Mouse Monoclonal Anti-Human CD57/HNK-1 (mouse IgM isotype) is derived from the VC1.1 hybridoma produced by the fusion of mouse myeloma cells and splenocytes from BALB/c mice immunized with a homogenate of cat cerebral cortical area 17.[1,2] The isotype is determined using the ImmunoType™ Kit (Cat. No. ISO-1) and by a double diffusion immunoassay using Mouse Monoclonal Antibody Isotyping Reagents (Cat. No. ISO-2).

Monoclonal anti-Human CD57/HNK-1 antibody may be used for the identification, quantification and monitoring of NK cells and T-cell subsets in peripheral blood, biological fluids, lymphoid organs, and other tissues; the studies of neural tissue glycoproteins and glycolipids and the 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).

Monoclonal anti-Human CD57/HNK-1 antibody recognizes the CD57/HNK-1, 110 kDa human myeloid cell associated surface glycoprotein. CD57/HNK-1 is expressed on a subpopulation (15-20%) of 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 also detected in lymphoid tissues, certain neuronal subsets, peripheral nerve Schwann cells, and normal, benign, and malignant prostate. Neurofibromas, schwannomas, traumatic neuromas, and small cell lung carcinomas frequently express HNK-1.[1-7] CD57/HNK-1 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 immunoglobulins superfamily molecules.[8] The epitope recognized by clone VC1.1 is an N-linked carbohydrate which is present in a variety of glyco-proteins, proteoglycans and in some glycolipids.[2-5] It is resistant to formalin fixation and paraffin embedding. VC1.1 antibody and the HNK-1 (Leu7)[9] 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 antibodies are reactive with the same set of polypeptides (including N-CAM and MAG) in Western blotting. In addition, both exhibit identical immunohistochemical staining patterns in neuronal subsets in cerebral cortex, retina, peripheral nerve Schwann cells, lymphoid tissues, and normal, benign and malignant prostate.

Reagents

The product is provided as purified antibody (200 µg/mL) in 0.01M phosphate buffered saline, pH 7.4, containing 1% BSA and 0.1% sodium azide as a preservative.

Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

Store at 2-8 °C. Do not freeze.

If slight turbidity occurs upon prolonged storage, clarify the solution by centrifugation before use.

Product Profile

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

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

It is advisable to run the appropriate negative controls. Negative controls establish background fluorescence and non-specific staining of the primary and secondary antibodies. The ideal negative control reagent is a mouse monoclonal or myeloma protein. It should be isotype-matched to the primary antibody, not specific for human cells and of the same concentration as the primary 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.

For fluorescence analysis of cells with Fc receptors, the use of isotype matched negative control is mandatory. In some systems it may be necessary to incubate the monoclonal antibody and cells (at step 4) in 10-20% normal serum from the second antibody host species in order to decrease non-specific staining with the conjugated second antibody.

Procedure

Indirect Immunofluorescent Staining

Reagents and Materials Needed but Not Supplied

- Whole human blood collected by standard clinical blood evacuation tubes with EDTA, ACD-A, or heparin anticoagulant OR
- Human cell suspension (Example: peripheral blood mononuclear cells isolated on Histopaque®, Cat. No. 1077-1.
- Diluent: 0.01M phosphate buffered saline (PBS), pH 7.4, containing 1% BSA and 0.1% NaN_3, prechilled to 4 °C
- Fluorochrome (FITC, PE, or TRITC) conjugated anti-mouse secondary antibody diluted to recommended, working dilution in diluent (Cat. No. F 2883 FITC Sheep anti-Mouse IgG (whole molecule), F(ab')2 Fragment of Affinity Isolated Antibody is recommended). Aggregates in conjugates should be removed by centrifugation immediately prior to use.
- Isotype matched non-specific mouse immunoglobulin (negative control, Cat. No. M 5909)
- 12 x 75 mm test tubes
- Adjustable micropipette
- Refrigerated centrifuge
- Counting chamber
- Trypan blue (Cat. No. T 0776), 0.2% in 0.01 M phosphate buffered saline, pH 7.4
- Whole blood lysing solution
- Flow cytometer or fluorescent microscope

Procedure

Prechill medium, perform all staining steps on ice and in a refrigerated centrifuge (2-6 °C).

- Use 100 µL of whole blood OR
- Adjust cell suspension to 2 x 107 cells/mL in diluent. Cells should be > 90% viable as determined by dye exclusion (trypan blue). For each sample, add 50 µL or 1 x 106 cells per tube.
- Prepare dilutions of Monoclonal anti-Human CD57/HNK-1 in diluent to give a concentration of 20 µg/mL, for example. 1:10 dilution of antibody. The CD57/HNK-1 antibody is provided at 200 µg/mL.
- Add 50 µL of diluted monoclonal antibody to tube(s) containing cells to be stained for example. 1 µg of monoclonal antibody per 1 x 106 cells in a final volume of 100 µL. Tap tube gently to mix. Incubate the cells on ice for 30 minutes.

Proper controls to be included for each sample are:

- An autofluorescence control: 50 µL diluent in place of secondary antibody and monoclonal antibody.
- A negative staining control: 50 µL isotype matched non-specific mouse immunoglobulin at a concentration of 20 µg/mL.
- Pellet cells by centrifugation at 500 x g, 2-6 °C, for 10 minutes.
- Remove supernatant by careful aspiration.
- Resuspend cells in 2 mL diluent.
- Pellet cells as in step 4 and repeat washing procedure (steps 5-6) twice.

- After the last wash, resuspend the cells in 50 µL of the fluorochrome conjugated second antibody (diluted in diluent containing BSA) at the recommended concentration, except for autofluorescence controls. Resuspend cells for autofluorescence controls in diluent. Incubate on ice for 30 minutes. Protect from light at this and all subsequent steps.

Note: If whole blood is used, use lysing solution after incubation according to manufacturer's instructions, then pellet and wash cells as in steps 4-6 twice and proceed to step 10.

- Centrifuge and wash as in steps 4-6 twice. Keep cells cold until analyzed.
- After last wash, resuspend cells in 0.5 mL of diluent and analyze in a flow cytometer according to manufacturer's instructions.

References

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