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ProductInformation

MONOCLONAL ANTI-HUMAN CD 13
Clone WM-47
Purified Mouse Immunoglobulin

Product Number **C 8589**

Product Description

Monoclonal Anti-Human CD13 (mouse IgG1 isotype) is derived from the WM-47 hybridoma produced by the fusion of mouse myeloma cells and splenocytes from BALB/c mice with human chronic myeloid leukemia cells.[1] The isotype is determined using Sigma Immuno-Type™ Kit (Product Code ISO-1) and by a double diffusion immunoassay using Mouse Monoclonal Antibody Isotyping Reagents (Product Code ISO-2).

The human CD13 antigen (also known as gp 150 and aminopeptidase N) is a 150 kDa cell surface glycoprotein that is expressed by committed granulocyte-monocyte progenitors (CFU-GM) and by cells of the granulocyte and monocyte lineages at all stages of differentiation.[1,2] It is also expressed by malignant counterparts of these cells. The majority of acute myeloid leukemia (AML), chronic myeloid leukemia in myeloid blast crisis, a smaller percentage of lymphoid leukemias, and myeloid cell lines express CD13.[2] CD13 is found on non-hematopoietic cells such as fibroblasts, endothelial cells, epithelial cells from renal proximal tubules and intestinal brush border, bone marrow stromal cells, osteoclasts, and cells lining the bile duct canaliculi.[2,3] It is present in a soluble form in blood plasma.[4] CD13 is identical to aminopeptidase N (APN) metalloprotease which catalyzes the removal of N terminal amino acids from peptides with a preference for neutral residues. It plays a role in the metabolism of biologically active peptides by various cells.[5] CD13 is thought to have a role in the control of growth and differentiation in both hematopoietic and epithelial cell systems, in the modulation of the biological activity of substances involved in phagocytosis and in bactericidal/tumoricidal activities. It also serves as a receptor for human coronaviruses (HCV).[6] Monoclonal Anti-Human CD13 is applicable in immunoprecipitation, immunohistochemistry, immunocytochemistry, and competitive binding assays.[1] The epitope recognized by the antibody is sensitive to routine formalin fixation and paraffin-embedding. The antibody reacts with neuroblastoma and certain gynecological carcinoma lines.[1] It weakly reacts with myeloid cells and epithelial cells in tonsil, with smooth muscle cells and connective tissue in spleen but not with liver, cerebellum and kidney.[1,7] The antibody is not inhibitory to HCV infection or to APN activity.[8,9] The WM-47 producing hybridoma was developed by E.J. Favaloro and coworkers in the department of Haematology, Institute of Clinical Pathology and Medical Research, Westmead Center, New South Wales, Australia.

Reagents

The product is provided as Protein A purified antibody in 0.01 M phosphate buffered saline, pH 7.4, containing 1% BSA and 15 mM sodium azide as a preservative.

Precautions and Disclaimer

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.

Storage/Stability

Store at 2 to 8 °C. Do not freeze. If slight turbidity occurs upon prolonged storage, clarify the solution by centrifugation before use.

Procedure

Indirect 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: 0.01 M phosphate buffered saline (PBS), pH 7.4, containing 1% BSA, and 0.1%NaN_3.

3. Fluorochrome (FITC, PE, or Quantum Red™) conjugated anti-mouse secondary antibody diluted to recommended working dilution in diluent (e.g. Product No. F 2883 FITC-Sheep Anti-Mouse IgG (whole molecule), F(ab´)$_2$ fragment of Affinity Isolated Antibody). Aggregates in conjugates should be removed by centrifugation immediately prior to use.
4. Isotype-matched, non-specific mouse immunoglobulin (negative control, Product No. M 5284).
5. 12 x 75 mm test tubes.
6. Adjustable micropipette.
7. Centrifuge.
8. Counting chamber.
9. 0.2% Trypan blue (Product No. T 0776) in 0.01 M phosphate buffered saline, pH 7.4.
10. 2% paraformaldehyde in PBS.
11. Whole blood lysing solution.
12. 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 5 µl of monoclonal antibody to tube(s) - containing cells to be stained. Vortex tube gently. Incubate the cells at room temperature (18 to 22 °C) for 30 minutes.
3. Proper controls to be included for each sample are:
   a. Autofluorescence control: 5 µl Diluent in place of monoclonal antibody.
   b. Negative staining control 1: 5 µl isotype-matched non-specific mouse immunoglobulin (Product No. M 5284) at the same concentration as test antibody.
4. After 30 minutes, 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 2 ml of Diluent.
8. Repeat washing procedure (steps 4-6).
9. After the second wash, resuspend the cells in 100 µl of the fluorochrome conjugated secondary - antibody at the recommended concentration. For the autofluorescence control, add 100 µl of diluent. Incubate at room temperature (18 to 22 °C) for 30 minutes. Protect from light at this and all subsequent steps.
   a. If whole blood is used, use lysing solution after incubation according to manufacturer's instructions, then proceed to Step 8.
   b. If a mononuclear cell suspension is used, proceed to Step 8.
10. Add 2 ml of Diluent to all tubes.
11. Wash as in steps 4-6 twice.
12. After last wash, resuspend cells in 0.5 ml of 2% paraformaldehyde and analyze in a flow cytometer according to manufacturer's instructions.

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 cells in 10-20% normal serum from the second antibody host species (at step 2 before adding monoclonal antibody) in order to decrease non-specific staining with the conjugated second antibody.

Product Profile

Monoclonal Anti-Human CD13 recognizes the human CD13 antigen expressed on the majority of peripheral blood monocytes and granulocytes and on endothelial cells.

Monoclonal Anti-Human CD13 is a homogenous population of antibody molecules which may be used for:

1. Detection and enumeration of myeloid cells in blood and tissue frozen sections and cell smears in health and disease.
2. Studies of immature myelopoiesis.
3. Typing AML and myelodysplastic syndromes.

When assayed by flow cytometric analysis (with a FACScan flow cytometer) using 5 µl of the antibody to stain 1 x 10^6 cells or 100 µl whole blood, 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.

References

1. Favaloro, E.J., et al., Br. J. Haematol., **69**, 163 (1988).
2. Hogg, N., et al., in: Leucocyte Typing III, pp 576, A.J. McMichael et al., (eds), Oxford University Press (1987).
3. Favaloro, E.J., et al., Br. J. Hematol., **74**, 385 (1990).
4. Favaloro, E.J., et al., Exp. Hematol., **21**, 1695 (1993).
5. Look, A.T., et al., J. Clin. Invest., **83**, 1299 (1989).
6. Yeager, C.L., et al., Nature, **357**, 420 (1992).
7. Favaloro, E.J., et al., Immunol. Cell. Biol., **69**, 253 (1991).
8. Leucocyte Typing V (code MA192), pp 771, S.F. Schlossman, et al., (eds.), Oxford University Press (1995).
9. Favaloro, E.J., et al., Clin. Chim. Acta, **220**, 81 (1993).

JWM/KMR 04/02