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ProductInformation

MONOCLONAL ANTI-HUMAN CD71
Clone DF 1513
Purified Mouse Immunoglobulin

Product Number **C 2063**

Product Description

Monoclonal Anti-Human CD71 (mouse IgG1 isotype) is derived from the DF 1513 hybridoma produced by the fusion of mouse myeloma cells and splenocytes from a Balb/c mouse immunized with the KG1 acute myeloid leukemia cell line. The isotype is determined using Sigma ImmunoType™ Kit (Product Code ISO-1) and by a double diffusion immunoassay using Mouse Monoclonal Antibody Isotyping Reagents (Product Code ISO-2).

The CD71[1] (T9 antigen, Transferrin Receptor) is a 95 kDa transmembrane, homodimeric glycoprotein with the two chains linked via disulfide bonds. The main function of CD71 is binding transferrin and the internalization of the complex for delivery of iron. Its expression correlates with DNA synthesis and proliferative activity. The antigen is expressed by virtually all permanent cell lines regardless of their cell lineage. It is present on T and B cells activated by antigens and mitogens, hemopoietic progenitor cells, reticulocytes (subset), macrophages, epithelioid cells, giant cells, osteoblasts, brain endothelium, germ cells and various malignant cells. Its expression is very low on resting lymphocytes or thymocytes and it is lacking in mature red blood cells, polymorphonuclear cells and monocytes. A truncated soluble CD71 is present in serum. The CD71 antibody can be used in flow cytometry and to stain rapidly proliferating cells in frozen tissue sections.[2,3]

Monoclonal Anti-Human CD71 antibody may be used for:

1. Identification of proliferating neoplastic smears, cytospins in cell suspensions and frozen sections.
2. Determination of activation state of T and B lymphocytes.
3. Immunohistochemical characterization of leukemias and lymphomas.
4. Studies of erythropoiesis.

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.

Reagent

The product is provided as purified antibody at approximately 200 µg/ml in 0.01 M phosphate buffered saline, pH 7.4, containing 1% BSA with 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 hazards and safe handling practices.

Storage/Stability

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

Procedure for 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 Code 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 Code M 5284)

5. 12 x 75 mm test tubes
6. Adjustable micropipet
7. Centrifuge
8. Counting chamber
9. 0.2% Trypan blue (Product Code 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 to mix. Incubate the cells at room temperature (18–22 °C) for 30 minutes.
   Proper controls to be included for each sample are:
   a . Autofluorescence control: 5 µl diluent in place of monoclonal antibody.
   b. Negative staining control: 5 µl isotype-matched non-specific mouse immunoglobulin at the same concentration as test antibody.
3. After 30 minutes, add 2 ml of diluent to all tubes.
4. Pellet cells by centrifugation at 500 x *g* for 10 minutes.
5. Remove supernatant by careful aspiration.
6. Resuspend cells in 2 ml diluent.
7. Repeat washing procedure (steps 4-6) twice.
8. After the last 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 (18-22 °C) 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.
9. Centrifuge and wash as in steps 4-6 twice.
10. After last wash, resuspend cells in 0.5 ml of diluent or 2% paraformaldehyde (if cells are stored before analyzing) 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
A working dilution of 1:10 is obtained by indirect immunohistology using frozen human tonsil sections.

References

1. Trowbridge S.I., et al., Proc. Natl. Acad. Sci., **78**, 3039 (1981).
2. Iacopetta, B., et al., J. Histochem. Cytochem., **31**, 336 (1983).
3. Leucocyte Typing IV, Oxford University Press, pp 455, 865, 908, 911, 920, 980.

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