MONOCLONAL ANTI-HUMAN CD71 (TRANSFERRIN RECEPTOR)
CLONE DF 1513
FITC Conjugate
Purified Mouse Immunoglobulin

Product No. **F8027**

Monoclonal Anti-Human CD71 (Transferrin Receptor) (mouse IgG1 isotype) is derived from the hybridoma produced by the fusion of mouse myeloma cells and splenocytes from mice immunized with K-G1 cultured cells. The isotype is determined using Sigma Immunotype™ Kit (Sigma Stock No. ISO-1) and by a double diffusion assay using Mouse Monoclonal Antibody Isotyping Reagents (Sigma Stock No. ISO-2). The product is prepared by conjugation of fluorescein isothiocyanate (FITC) Isomer I to purified CD71 monoclonal antibody. The conjugate is then purified by gel filtration to remove unbound FITC, no free FITC is detectable. The conjugate is provided (200 µg/ml) in 0.01 M phosphate buffered saline, pH 7.4, containing 1% BSA with 15mM sodium azide (see MSDS)* as a preservative.

Description
The CD71[1] (T9 antigen, Transferrin Receptor) is a 95 kD transmembrane homodimeric glycoprotein with the two chains linked via disulfide bonds. The main function of CD71 is binding transferrin and internalization of the complex for delivery of iron. Its expression correlates with DNA synthesis and cell proliferation. The antigen is expressed by virtually all permanent cell lines regardless of their 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]

Performance
When assayed by flow cytometric analysis, using 10 µl 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.

F/P Molar Ratio: 3.0-5.0

Uses
Monoclonal Anti-Human CD71 may be used for:
1. Identification of proliferating neoplastic smears, cytospins, 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.

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 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.

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.

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® (Sigma Product No. 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, non-specific mouse immunoglobulin (negative control, Sigma Product No. F6397).
4. 12 x 75 mm test tubes.
5. Adjustable micropipet.
6. Centrifuge.
7. Counting chamber.
8. Trypan blue (Sigma 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 (Sigma Product No. F6397) 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

1. Trowbridge, S.I., et al., Proc. Natl. Acad. Sci. USA, **78**, 3039 (1981).
2. Iacopetta, B., et al., J. Histochem. Cytochem., **31**, 336 (1983).
3. Leucocyte Typing IV, Knapp, W., et al., (eds.), Oxford University Press, Oxford, pp 455, 865, 908, 911, 920, 980 (1989).

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