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ProductInformation

MONOCLONAL ANTI-HUMAN CD19
FITC Conjugate
Purified Mouse Immunoglobulin
Clone SJ25-C1

Product Number **F3899**

Product Description

Monoclonal Anti-Human CD19 antibody (mouse IgG1 isotype) is derived from the SJ25-C1 hybridoma produced by the fusion of mouse myeloma cells and splenocytes from BALB/c mice immunized with the NALM-1 human cell line. The product is prepared by conjugation of fluorescein isothiocyanate (FITC) to the purified CD19 monoclonal antibody. The conjugate is then purified by gel filtration to remove unbound FITC, no free FITC is detectable.

Monoclonal Anti-Human CD19 recognizes the CD19 (90-95 kDa) glycoprotein antigen, which is broadly distributed in the B cell lineage.[1] CD19 defines a pan-B antigen, which is expressed from the earliest stages of B-progenitor development, but is lost on terminal differentiation to plasma cells.[2] It may also be present on some early myeloid progenitors, particularly those of the monoblastic type.[3] The CD19 antigen is expressed on approximately 12% of peripheral blood lymphocytes.[4] It appears to be expressed on myeloid leukemia cells, particularly those of monocytic lineage.[5] Leukemia phenotype studies have demonstrated that the earliest and broadest B cell restricted antigen is the CD19 antigen. The receptor for CD19 is an important functional regulator of normal and malignant B cell proliferation.[6] It is expressed in all B cell precursor leukemias. Recent cDNA cloning of CD19 has shown significant homology in the cytoplasmic domain of CD19 with the int-1 oncogene.[6]

Reagents

The product is provided as purified antibody conjugate (200 µg/ml) 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 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. Protect from prolonged exposure to light. If slight turbidity occurs upon prolonged storage, clarify the solution by centrifugation before use.

Procedure

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

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.

Product Profile

When assayed by flow cytometric analysis, using 10 µl of the conjugate 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.

FITC Conjugated Monoclonal Anti-Human CD19 may be used for:
1. Identification of pan B cells and B progenitors.
2. Identification of germinal center B cells.

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.

References

1. Ling, N., et al., Leucocyte Typing III, 302 (1987).
2. Dorken, B., et al., Leucocyte Typing IV, 34 (1989).
3. Umiel, K., et al., Leucocyte Research, **10**, (1986).
4. Bofill, M., et al., Clin. Exp. Immunol., **88**, 243 (1992).
5. Campos, L., et al., Leucocyte Typing III, 470 (1987).
6. Campos, L., et al., Leucocyte Typing III, 473 (1987).

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