



**MONOCLONAL ANTI-HUMAN CD19
R-PHYCOERYTHRIN CONJUGATE**
Purified Mouse Immunoglobulin Clone SJ25-c1

Product Number **P 7437**

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 R-Phycoerythrin (R-PE) to purified CD19 monoclonal antibody. The conjugate is then purified by gel filtration to remove unbound R-PE; no free R-PE is detectable.

Monoclonal Anti-Human CD19 recognizes the CD19 (90-95kD) 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 (20 µg/ml) in 0.01 M phosphate buffered saline, pH 7.4, containing 1% BSA and 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.

Product Information

Storage/Stability

Store at 0-5 °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

- 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)).
- Diluent: 0.01 M phosphate buffered saline (PBS), pH 7.4, containing 1% BSA, and 0.1% NaN₃.
- R-PE conjugated, isotype-matched, non-specific mouse immunoglobulin (negative control, Product No. P 4685).
- 12 x 75 mm test tubes.
- Adjustable micropipet.
- Centrifuge.
- Counting chamber.
- Trypan blue (Product No. T 0776), 0.2% in 0.01 M phosphate buffered saline, pH 7.4.
- 2% paraformaldehyde in PBS.
- Whole blood lysing solution.
- Flow cytometer.

Procedure

- a. Use 100 µl of whole blood **or**
- b. Adjust cell suspension to 1 x 10⁷ 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 10⁶ cells per tube.
- Add 10 µl of conjugate 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. An autofluorescence control: 10 μ l diluent in place of monoclonal antibody followed by steps 3 - 8.
 - b. A negative staining control: 10 μ l of R-PE conjugated, isotype-matched non-specific mouse immunoglobulin at the same concentration as test antibody followed by steps 3 - 8.
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 3-6) twice. Note: If whole blood is used, use lysing solution after incubation according to manufacturer's instructions, then repeat steps 3-6 twice, and proceed to step 8.
 8. 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 (at step 2 before adding monoclonal antibody) in 10-20% normal serum from the second antibody host species in order to decrease non-specific staining with the conjugated second antibody.

Product Profile

When assayed by flow cytometric analysis, using 10 μ l of the conjugate to stain 1×10^6 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.

R-PE 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.

References

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