Product No. C-8080
Lot 045H4844

Monoclonal Anti-Human CD20
Purified Mouse Immunoglobulin
Clone B9E9

Monoclonal Anti-Human CD20 (mouse IgG2a isotype) is derived from the B9E9 hybridoma produced by the fusion of mouse myeloma cell line NS-1 and splenocytes from an immunized mouse. The human lymphoblastoid cell line Daudi was used as immunogen.[1] The isotype is determined using Sigma ImmunoType™ Kit (Sigma Stock No. ISO-1) and by a double diffusion immunoassay using Mouse Monoclonal Antibody Isotyping Reagents (Sigma Stock No. ISO-2). The product is provided as purified antibody (200 µg/ml) in 0.01 M phosphate buffered saline, pH 7.4, containing 1% BSA with 0.1% sodium azide (see MSDS)* as a preservative.

Description

Monoclonal Anti-Human CD20 recognizes the B cell-specific antigen, human CD20, expressed by pre-B cells, resting and activated B cells but not by pro-B cells, plasma cells or myeloma cells. CD20 is a 33/35/37 kD membrane-embedded, unglycosylated, phosphoprotein with four membrane-spanning domains as well as serine and threonine rich cytoplasmic N-terminal and C-terminal domains. Only a minor portion of the molecule consisting of one short and one longer loop is exposed on the cell surface.[2,3,4] CD20 exists on the cell surface as a homo-oligomeric complex. CD20 is expressed on some non T-ALL cells and possibly on follicular dendritic cells but not on monocytes, macrophages, neutrophils, red blood cells or platelets. In normal lymph nodes and tonsils, CD20 is detected on B cells in germinal centers while lower density expression is manifested on cells in the mantle zone.[5] CD20 is associated with transmembrane Ca^{2+} conductance and is involved in the regulation of B cell proliferation and differentiation. While CD20 is not phosphorylated in resting B cells, it is differentially phosphorylated in its cytoplasmic domains by ubiquitous kinases in activated cells or in B cell lines. Binding of many monoclonal antibodies to CD20 may cause generation of transmembrane signals that result in enhanced CD20 and other cellular proteins phosphorylation, induction of oncogene expression and increased expression of CD18, CD58 and MHC Class II antigens.[6] Other antibodies inhibit B cell proliferation and differentiation. In flow cytometric analysis, Anti-Human CD20 reacts dimly with T cells, thus requiring resetting the boundary between positive and negative cells to include only the brightly stained cells as positive. Monoclonal Anti-Human CD20 may be used in flow cytometry, immunohistochemistry and immunocytochemistry. The epitope recognized by the antibody is sensitive to routine formalin fixation and paraffin embedding procedures.

Working Dilution

A dilution of 1:10 was determined by indirect immunofluorescent staining of human tonsil frozen sections.

Performance

When assayed by flow cytometric analysis, 5 µl of the monoclonal antibody will stain 1 x 10^6 cells with a fluorescence intensity and percent positive that is similar to that observed with saturating amounts of monoclonal antibody.

Uses

Monoclonal Anti-Human CD20 may be used for:

1. Identification of normal, leukemic and lymphoma B cells in smears, cytospins, cell suspensions and frozen sections.
2. Enumeration of B lymphocytes in peripheral blood.
3. B cell isolation by sorting procedures.

Storage

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

In order to obtain best results in different preparations, it is recommended that each individual user determine their optimum working dilutions by titration assay.

*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.

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® (Sigma Stock No. 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. Sigma 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, Sigma Product No. M-5409).
5. 12 x 75 mm test tubes.
6. Adjustable micropipet.
7. Centrifuge.
8. Counting chamber.
9. 0.2% Trypan blue (Sigma 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 - 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 (Sigma Product No. M-5409) 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).
8. 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 - 22°C) for 30 minutes. Protect from light at this and all subsequent steps.
9. 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 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.

Quality Control

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.

References

1. Leucocyte Typing V, Schlossman, S., et al., (eds.), Oxford University Press, Oxford, pp 511, 513, 515 (1995).
2. Tedder, T., et al., J. Immunol., **141**, 4388 (1988).
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5. Ledbetter, J., and Clark, E., Human Immunology, **15**, 30 (1986).
6. Tedder, T., and Engel, P., Immunology Today, **15**, 450 (1994).

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