



Product Information

MONOCLONAL ANTI-HUMAN CD38

Clone HI157

Purified Mouse Immunoglobulin

Product No. C 1586

Product Description

Monoclonal Anti-Human CD38 (mouse IgG2a isotype) is derived from the HI157 hybridoma produced by the fusion of mouse myeloma cells and splenocytes from an immunized mouse. CEM T line cells were used as the immunogen.^{1,2} 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).

Monoclonal Anti-Human CD38 recognizes the human CD38 antigen mainly expressed by thymocytes, activated T cells and plasma cells.

CD38 (T10) is a single chain 46 kDa type II integral glycoprotein with a short N-terminal cytoplasmic tail.^{3,4} CD38 is highly expressed on thymocytes. It is also expressed by early cells of B and T lineages, NK cells, plasma cells, monocytes and macrophages and may be detected on cells from multiple myeloma, ALL (B and T) and some AML. In normal lymph nodes and tonsils the antigen is detected mainly on B cells in germinal centers and in plasma cells. The extracellular domain of the molecule shares a high homology sequence with Aplysia ADP ribosyl cyclase. CD38 functions as a multicatalytic ectoenzyme serving as ADP ribosyl cyclase, ADPR hydrolase and possibly NAD⁺ glycohydrolase, or as a surface receptor. Antibodies to CD38 potentiate many biological activities on lymphocytes such as cell activation, proliferation and adhesion. Monoclonal Anti-Human CD38 can be used in flow cytometry. It is valuable in studies of activated T cells in immunodeficiency diseases and in autoimmune diseases. The epitope recognized by the antibody is sensitive to routine formalin-fixation and paraffin-embedding procedures.

Monoclonal Anti-Human CD38 may be used for identification of normal and abnormal leukemic and lymphoma cells and plasma cells. It is also useful for studying B and T cell activation.

When assayed by flow cytometric analysis, using 5 µl of the antibody to stain 1 x 10⁶ 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.

In order to obtain best results, it is recommended that each user determine the optimal working dilution for individual applications by titration assay.

Reagents

The product is provided as (Protein A) purified antibody 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 sheet (MSDS) has been sent to the attention of the safety officer of your institution. Consult the MSDS for information regarding hazardous and safe handling practices.

Storage

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

- 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)).

Reagents and Materials Needed but Not Supplied (cont.)

2. Diluent: 0.01 M phosphate buffered saline (PBS), pH 7.4, containing 1% BSA and 0.1% NaN₃.
3. Fluorochrome (FITC, PE, or Quantum Red™) conjugated anti-mouse secondary antibody diluted to recommended working dilution in diluent (e.g. Product No. F2883 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 No. M 5409).
5. 12 x 75 mm test tubes.
6. Adjustable micropipet.
7. Centrifuge.
8. Counting chamber.
9. 0.2% Trypan blue (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 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.
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 1:
5 µl isotype-matched non-specific mouse immunoglobulin (Product No. M 5409) at the same concentration as test antibody.
3. After 30 minutes, add 2 ml of diluent to all tubes.
3. 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 10.
b. If a mononuclear cell suspension is used, proceed to Step 10.
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. Chunying Y., et al., J. Monoclonal Antibody (China) **9**, 56 (1993).
2. Leucocyte Typing V, Oxford University Press, p 249 (clone No. CD38.04) (1995).
3. Jackson, D., and Bell, J., J. Immunol., **144**, 2811 (1990).
4. Alessio, M., et al., J. Immunol., **145**, 878 (1990).

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