3050 Spruce Street
Saint Louis, Missouri 63103 USA
Telephone 800-325-5832 • (314) 771-5765
Fax (314) 286-7828
email: techserv@sial.com
sigma-aldrich.com

ProductInformation

Monoclonal Anti-Human CD45
Clone Bra-55
Purified Mouse Immunoglobulin

Product Number **C 7556**

Product Description

Monoclonal Anti-Human CD45 antibody (mouse IgG1 isotype) is derived from the Bra-55 hybridoma produced by the fusion of mouse myeloma cells and splenocytes from BALB/c mice immunized with the non-T, non-B CALLA positive, ALL cell line REH.[1-3] 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 CD45 antibody may be used for:

1. Identification, quantification, and monitoring of white blood cells and hematopoietic progenitor cells.
2. Characterization of leukemias and lymphomas.
3. Discrimination of hematopoietic neoplasms from other neoplasms.
4. Detection of infiltrating hematopoietic cells in tissues.
5. Inhibition or upregulation of various immunological functions.

Monoclonal Anti-Human CD45 antibody recognizes the CD45 human cell surface glycoproteins 180, 190, 205, and 220 kDa. CD45 is a family of single chain trans-membraneous glycoproteins consisting of at least four isoforms which share a common large intracellular domain. Their extracellular domains are heavily glycosylated. The different isoforms are produced by alternative messenger RNA splicing of three exons of a single gene on chromosome 1. CD45 is expressed on cells of the human hematopoietic lineage with the exception of mature red cells.[4, 5] It is not detected on differentiated cells of other tissues. It is likely that CD45 plays an important role in signal transduction. The intracellular domain of all members of the CD45 family display a cytoplasmic tyrosine phosphatase activity. Also, CD45 isoforms may form complexes with different membrane molecules such as CD2 on T cells. Monoclonal antibodies to CD45 are particularly valuable in immunohematology and immunohistology. The epitope recognized by CD45 monoclonal antibody (Bra-55) is sensitive to formalin fixation and paraffin embedding.

Reagent

Monoclonal Anti-Human CD45 is provided as purified antibody 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 hazardous and safe handling practices.

Storage/Stability

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

Procedure

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 (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, and 0.1% NaN_3.
3. Fluorochrome (FITC, PE, or TRITC) conjugated anti-mouse secondary antibody diluted to recommended working dilution in diluent (Product No. F 2883 FITC Sheep anti-Mouse IgG (whole molecule), $F(ab')_2$ Fragment of Affinity Isolated Antibody is recommended). Aggregates in conjugates should be removed by centrifugation immediately prior to use.
4. Isotype matched non-specific mouse immuno-globulin (negative control, Product No. M 5284).
5. 12 x 75 mm test tubes.
6. Adjustable micropipette.
7. Centrifuge.

8. Counting chamber.
9. Trypan blue (Product No. T 0776), 0.2% in 0.01 M phosphate buffered saline, pH 7.4.
10. 2% paraformaldehyde in 0.01 M phosphate buffered saline.
11. Whole blood lysing solution.
12. Flow cytometer or fluorescent microscope.

Procedure

1. a. Use 100 µl of whole blood **or**
   b. Adjust cell suspension to 2 x 10^7 cells/ml in diluent. Cells should be >90% viable as determined by dye exclusion (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. Tap 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: 5 µl diluent in place of monoclonal antibody followed by steps 3-8.
   b. A negative staining control: 5 µl isotype-matched non-specific mouse immunoglobulin at the same concentration as the 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 of diluent.
7. Pellet cells as in step 4 and repeat washing procedure (steps 5-6) twice.
8. After the last wash, resuspend the cells in 100 µl of the fluorochrome conjugated secondary antibody (diluted in diluent) at the recommended concentration, except for autofluorescence controls. For the autofluorescence controls, resuspend the cells in 100 µl diluent only. Incubate at room temperature (18-22 °C) for 30 minutes. Protect from light at this and all subsequent steps.
   Note: If whole blood is used, use lysing solution after incubation according to manufacturer's instructions, then pellet and wash cells as in steps 4-6 twice, and proceed to step 10.
9. Centrifuge and wash as in steps 4-6 twice.
10. After last wash, resuspend cells in 0.5 ml of diluent or 2% paraformaldehyde (if the cells are stored before analysis). Analyze in a flow cytometer according to manufacturer's instructions.
    Note: Keep cells cold until analyzed.

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 maybe necessary to incubate the monoclonal antibody and cells (at step 4) in 10-20% normal serum from the secondary antibody host species in order to decrease non-specific staining with the conjugated secondary antibody.

Product Profile

When assayed by flow cytometric analysis, using 5 µl of the antibody to stain 1 X 10^6 cells, 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.

References

1. Chorvath, B., et al., Neoplasma, **34**, 685 (1987).
2. Chorvath, B., et al., in Leukocyte Typing IV, Knapp, W. (ed.), p. 634, Oxford University Press (1989).
2. Chorvath, B., et al., Neoplasma, **35**, 495 (1988).
3. Dalchau, R., et al., Eur. J. Immunol., **16**, 993 (1986).
4. Pinkus, G.S., in Advances in Immunohistochemistry, DeLellis, R.A. (ed.), p. 261, Raven Press (1988).

KAA 07/03