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# ProductInformation

**MONOCLONAL ANTI-HUMAN CD45**
**FITC CONJUGATE**
**Clone Bra-55**
Purified Mouse Immunoglobulin

Product Number **F4149**

## Product Description

Monoclonal Anti-Human CD45 (mouse IgG1 isotype) is derived from the hybridoma produced by the fusion of mouse myeloma cell line NS-1 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 the Sigma ImmunoType™ Kit (Product Code ISO-1) and by a double diffusion immunoassay using Mouse Monoclonal Antibody Isotyping Reagents (Product Code ISO-2). The product is prepared by conjugation of fluorescein isothiocyanate (FITC) Isomer I to purified CD45 monoclonal antibody. The conjugate is then purified by gel filtration to remove unbound FITC. No free FITC is detectable.

Monoclonal Anti-Human CD45 antibody recognizes the CD45 human cell surface glycoproteins of 180, 190, 205, and 220 kDa. CD45 is a family of single chain transmembraneous 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 displays 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 this CD45 monoclonal antibody (Bra-55) is sensitive to formalin fixation and paraffin embedding.

## Reagents

The product is provided as purified immunoglobulin 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) 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. Protect from prolonged exposure to light. If slight turbidity occurs upon prolonged storage, clarify the solution by centrifugation before use.

## Procedure

Direct Immunogluorescent Staining

Reagents and Material 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 7 (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. FITC conjugated, isotype-matched, non-specific mouse immunoglobulin (negative control, Product No. F6397).
4. 12 x 75 mm test tubes.
5. Adjustable micropipette.
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 (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 to 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. Then proceed to step 4.
   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 antibody 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.

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.

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

**References**


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