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Product Information

Monoclonal Anti-CD45-PE, clone BRA-55
produced in mouse, purified immunoglobulin

Catalog Number **P7687**

Product Description

Monoclonal Anti-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 by a double diffusion immunoassay using Mouse Monoclonal Antibody Isotyping Reagents, Catalog Number ISO2. The product is prepared by conjugation of R-Phycoerythrin (PE) to purified CD45 monoclonal antibody. The conjugate is then purified by gel filtration to remove unbound PE. No free antibody is detectable.

Reagents

The product is provided as purified antibody (150 µg/ml) in 0.01 M phosphate buffered saline, pH 7.4, containing 1% BSA and 15 mM sodium azide as a preservative.

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Description

Monoclonal Anti-CD45 antibody recognizes the CD45 human cell surface glycoproteins 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 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.

Performance

When assayed by flow cytometric analysis, using 10 µl of the conjugate to stain 1 X 10^6 cells, a fluorescence intensity and percent population positive is observed similar to that obtained with saturating monoclonal antibody levels.

A_{567}/A_{280}: 1 to 4

Uses

Monoclonal Anti-CD45-PE 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.

Storage

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 for Direct 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®, Catalog Number 10771.
2. Diluent: 0.01 M phosphate buffered saline (PBS), pH 7.4, containing 1% BSA and 0.1% NaN_3.
3. PE conjugated, isotype-matched, non-specific mouse immunoglobulin (negative control, Catalog No. P4685).
4. 12 x 75 mm test tubes.
5. Adjustable micropipet.
6. Centrifuge.
7. Counting chamber.
8. Trypan blue, Catalog No. 302643, 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 (e.g., 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 – 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 PE conjugated, isotype-matched non-specific mouse immunoglobulin (Catalog No. P4685) 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.
   c. If a mononuclear cell suspension is used, proceed to Step. 4.
3. 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 0.5 ml of 2% paraformaldehyde. 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 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.

References

1. Chorvath, B., et al., Neoplasma, **34**, 685 (1987).
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3. Sedlak, J., et al., Neoplasma, **35**, 495 (1988).
4. Dalchau, R., et al., Eur. J. Immunol., **16**, 993 (1986).
5. Pinkus, G.S., in Advances in Immunohistochemistry, DeLellis, R.A. (ed.), p. 261, Raven Press (1988).

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