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ProductInformation

ANTI-HUMAN CD45-FITC/CD14-PE
DUAL-TAG™
Clones BRA-55/UCHM-1

Product Number **F 8527**

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. The product is prepared by the 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 CD14 (mouse IgG2a 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 human thymocytes followed by peripheral blood T cells. The product is prepared by conjugation of R-Phycoerythrin (PE) with purified CD14 monoclonal antibody. The conjugate is then purified by gel filtration to remove unbound PE and antibody, no free PE or free antibody is detectable.

Monoclonal Anti-Human CD45 antibody recognizes the CD45 human cell surface glycoprotein of 180, 190, 205, and 220 kDa. CD45 is a family of single chain transmembrane 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 hematopoietic lineage with the exception of mature red cells. 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 may form complexes with different membrane molecules such as CD2 on T cells. Monoclonal antibodies to CD45 are particularly useful in immunohematology and immunohistology. The epitope recognized by this CD45 monoclonal antibody (BRA-55) is sensitive to formalin fixation and paraffin embedding. Monoclonal Anti-Human CD14 recognizes the CD14 monocyte surface glycoprotein, a phosphoinositol 55 kDa molecule. This antigen is expressed on most peripheral blood monocytes and tissue macrophages, it is also present in cell cytoplasm and may be found cell free in urine and serum. The epitope recognized by this clone is sensitive to routine formalin fixation and paraffin embedding. Cryostat sections post fixed in formalin can also be stained.

Reagents

The two conjugates are provided as a pre-titered solution in 0.01 M phosphate buffered saline, pH 7.4, containing 1% BSA with 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. Protect from prolonged exposure to light. If slight turbidity occurs upon prolonged storage, clarify the solution by centrifugation before use.

Procedure

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® (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 and PE conjugated, isotype-matched, non-specific mouse immunoglobulins (negative control, FITC Mouse IgG1/PE Mouse IgG2a, Product No. F 0528).
4. 12 x 75 mm test tubes.
5. Adjustable micropipette.

6. Centrifuge.
7. Counting chamber.
8. Trypan blue (Product No. T 0776), 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 20 µl of conjugates 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: 20 µl of Diluent in place of monoclonal antibodies, followed by steps 3 - 7.
   b. A negative staining control: 20 µl of FITC and PE conjugated, isotype-matched, non-specific mouse immunoglobulins at the same concentration as test antibody (Product No. F 0528), followed by steps 3 - 7.
3. a. If whole blood is used, use lysing solution after incubation 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. Proper color compensation is important for unbiased data interpretation. Cell samples stained with the corresponding single reagents of the pair may be used as controls for adjusting compensation. Alternatively, microbead standards may be used (Flow Cytometry Compensation Kit, Product Code COMP-1)

It is advisable to run the appropriate negative controls. Negative controls establish background fluorescence and non-specific binding of the antibodies. The ideal negative control reagent is a combination of a FITC- and PE-conjugated mouse monoclonal or myeloma proteins which have no reactivity with human cells. It should be isotype-matched to the antibodies in the DUAL TAG antibody reagent and of the same concentration and F/P molar ratio as the DUAL TAG antibody reagent. 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 20 µl of the antibody to stain 1 x 10^6 cells, a fluorescence intensity for each antibody conjugate is observed similar to that obtained with saturating monoclonal antibody levels of each conjugate in single color flow cytometry.

Anti-Human CD45-FITC/CD14-PE DUAL-TAG may be used for:

1. Differentiation of leucocyte subpopulations (lymphocytes, monocytes and granulocytes) by immunophenotypes (i.e. gating by immunophenotypes).
2. Verification of the accuracy of leucocyte gates set by light scatter characteristics.
3. Measurement of the degree of cross contamination of light scatter leucocyte gates by inappropriate subpopulation(s).

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