Anti-Human CD5-FITC/CD19-R-PE
DUAL-TAG™
Clones: UCHT-2/SJ25-C1

Product No. **F1278**
Lot 025H88061

Monoclonal Anti-Human CD5, clone UCHT-2 (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 human T cells. The product is prepared by conjugation of fluorescein isothiocyanate (FITC) Isomer I to purified CD5 monoclonal antibody. The conjugate is then purified by gel filtration to remove unbound FITC, no free FITC is detectable.
F/P Molar Ratio: 6.2
Concentration: 25 µg/ml

Monoclonal Anti-Human CD19, clone SJ25-C1 (mouse IgG1 isotype) is derived from the hybridoma produced by the fusion of mouse myeloma cells and splenocytes from BALB/c mice immunized with the NALM-1 human cell line. The product is prepared by conjugation of R-Phycoerythrin (PE) to purified CD19 monoclonal antibody. The conjugate is then purified by gel filtration to remove unbound PE, no free PE is detectable.
A_{567}/A_{280}: 2.1
Concentration: 30 µg/ml

The conjugates are provided as a pre-titered solution in 0.01 M phosphate buffered saline, pH 7.4, containing 1% BSA with 0.1% sodium azide (see MSDS)* as a preservative.

Description
Monoclonal Anti-Human CD5 recognizes the CD5 human lymphocyte surface antigen glycoprotein (molecular weight 67 kD). This antigen is present on more than 90% of the normal peripheral blood T cell population. The antibody stains medullary thymocytes strongly and weakly stains cortical thymocytes. A subpopulation of normal B cells and the majority of B-CLL cells are stained by this antibody. Monoclonal Anti-Human CD5 does not stain monocytes, granulocytes or NK cells. The epitope recognized by this clone is sensitive to routine formalin fixation and paraffin embedding.

Monoclonal Anti-Human CD19 recognizes the CD19 (90-95 kD) glycoprotein antigen, which is broadly distributed in the B cell lineage. CD19 defines a pan-B antigen which is expressed from the earliest stages of B progenitor development, but is lost on terminal differentiation to plasma cells. It may be present on some early myeloid progenitors, particularly those of the monoblast type. The CD19 antigen is expressed on approximately 12% of peripheral blood lymphocytes. It appears to be expressed on myeloid leukemia cells, particularly those of monocyte lineage. Leukemia phenotype studies have demonstrated that the earliest and broadest B cell restricted antigen is the CD19 antigen. The receptor for CD19 is an important functional regulator of normal and malignant B cell proliferation. It is expressed in all B cell precursor leukemias. Recent cDNA cloning of CD19 has shown significant homology in the cytoplasmic domain of CD19 with the int-1 oncogene.

Performance
When assayed by flow cytometric analysis using 20 µl of the antibodies 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.

Uses
Anti-Human CD5-FITC/CD19-PE DUAL TAG may be used for characterizationof leukemias and lymphomas.

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.

* 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.

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® (Sigma Product No. 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 IgG1, Sigma Product No. F0403).
4. 12 x 75 mm test tubes.
5. Adjustable micropipet.
6. Centrifuge.
7. Counting chamber.
8. Trypan blue (Sigma 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 (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 - 22°C) for 30 minutes. Proper controls to be included for each sample are:
   a. An autofluorescence control: 20 µl 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 (Sigma Product No. F0403), followed by steps 3 - 7.
3. a. If whole blood is used, use lysing solution after incubation according to manufacturer's instructions.
   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, Sigma Stock No. COMP-1)

Quality Control

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.

Selected References

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