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ProductInformation

MONOCLONAL Anti-Human CD14
CLONE UCHM-1 BIOTIN CONJUGATE
Purified Mouse Immunoglobulin

Product No. **B 0781**

Product Description

Monoclonal Anti-Human CD14 (mouse IgG2a isotype) is derived from the UCHM-1 hybridoma produced by the fusion of mouse myeloma cell line and splenocytes from BALB/c mice immunized with circulating human thymocytes followed by peripheral blood T cells.[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 ε-amino caproyl biotin to purified CD14 monoclonal antibody.

Monoclonal Anti-Human CD14 antibody recognizes the CD14, 55 kDa human cell surface glycoprotein. CD14 is a phosphatidylinositol linked single chain glycoprotein. It is found on most monocytes, is expressed by some macrophages and is weakly expressed by some granulocytes. It is also detectable in Langerhans cells, follicular dendritic cells and histiocytes. CD14 is also found in cell cytoplasm and in urine. The epitope recognized by monoclonal UCHM-1 is sensitive to routine formalin fixation and paraffin embedding.

Reagent

The conjugate is provided as purified immunoglobulin (200 µg/ml) in 0.01 M phosphate buffered saline, pH 7.4, containing 1% BSA and 15mM 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.

Performance

When assayed by flow cytometric analysis, using 10 µl of the antibody per 1 x 10^6 cells and ExtrAvidin®-FITC Conjugate (Product No. E2761), 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.

Uses

Biotin Monoclonal Anti-Human CD14 antibody may be used for:

1. Detection and enumeration of monocytes in bone marrow, peripheral blood, other tissues and body fluids.
2. Differentiation of myeloid cells.

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.

Storage

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

Procedure for Indirect Immunofluorescent Staining using Biotinylated Primary Antibodies

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. Fluorochrome (FITC, PE, or Quantum Red™) conjugated avidin derivative diluted to recommended working dilution in diluent. Appropriate products for use are ExtrAvidin®-FITC (Product No. E 2762), Streptavidin-FITC (Product No. S 3402), Streptavidin-PE (Product No. S 3762), or Streptavidin-Quantum Red™ (Product No. S 2899).
4. 12 x 75 mm test tubes.
5. Adjustable micropipet.
6. Centrifuge.
7. Counting chamber.
8. 0.2% Trypan blue (Product No. T 0776) in 0.01 M phosphate buffered saline, 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 biotinylated monoclonal antibody to tube(s) containing cells to be stained. Vortex tube gently to mix. Incubate the cells at room temperature (18 – 22 °C) for 30 minutes.
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 diluent.
7. Repeat washing procedure (steps 4-6) twice.
8. After the last wash, resuspend the cells in 100 µl of the fluorochrome conjugated avidin derivative at the recommended concentration. For the autofluorescence control, add 100 µl of diluent. Incubate at room temperature (18 - 22 °C) for 30 minutes. Protect from light at this and all subsequent steps.
9. a. If whole blood is used, use lysing solution after incubation according to manufacturer's instructions, then proceed to Step 10.
   b. If a mononuclear cell suspension is used, proceed to Step 10.
10. Centrifuge and wash as in steps 4 - 6 twice.
11. After last wash, resuspend cells in 0.5 ml of diluent or 2% paraformaldehyde (if cells are stored before analyzing) and analyze in a flow cytometer according to manufacturer's instructions.

References

1. Hogg, N.H., et al., Immunology, **53**, 753 (1984).
2. Linch, D.C., et al., Blood, **63**, 566 (1984).
3. Khin, Y., et al., Int. J. Cancer, **36**, 433, (1985).

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