3050 Spruce Street
Saint Louis, Missouri 63103 USA
Telephone 800-325-5832 • (314) 771-5765
Fax (314) 286-7828
email: techserv@sial.com
sigma-aldrich.com

ProductInformation

Monoclonal Anti-Human CD56 (NKH-1),
Clone C5.9
FITC Conjugate

Product Number **F 0301**

Product Description

Monoclonal Anti-HumanCD56 (NKH-1) (mouse IgG2b-kappa isotype) is derived from the C5.9 hybridoma produced by the fusion of mouse myeloma cells and splenocytes from a mouse immunized with cloned human NK cell line termed JT3. The antibody is purified from ascites fluid using Protein A chromatography. The product is prepared by conjugation of fluorescein isothiocyanate (FITC) Isomer I to purified CD56 monoclonal antibody.

Monoclonal Anti-Human CD56 (NKH-1) FITC Conjugate recognizes CD56 (150 kDa) by flow cytometry.

CD56 is expressed on approximately 10 % to 25 % of human peripheral blood lymphocytes. CD56 is expressed on human peripheral blood natural killer cells, representing a pan NK-cell antigen. It is also expressed on non-MHC-restricted cytotoxic T cells.

Uses

Monoclonal Anti-Human CD56 may be used for the study of:

1. Resting and activated NK cells.
2. Non-MHC-restricted cytotoxic T cells.
3. T cell differentiation in early thymocytes.

Reagent

Monoclonal Anti-Human CD56, FITC Conjugate is supplied as a solution in 0.01 M phosphate buffered saline, pH 7.4, containing 0.2 % gelatin and 0.08 % sodium azide.

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 hazards and safe handling practices.

Storage/Stability

For continuous use, store at 2 °C to 8 °C. Working dilution samples should be discarded if not used within 12 hours.

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: 0.01M 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, Sigma Product No. F6647)
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 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 conjugate to tube(s) containing cells to be stained. Vortex tube gently to mix. Incubate the cells at room temperature (18 °C 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 - 8.

b. A negative staining control: 10 µl of FITC conjugated, isotype-matched non-specific mouse immunoglobulin at the same concentration as test antibody followed by steps 3 - 8.

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 3-6) twice.
   Note: If whole blood is used, use lysing solution after incubation according to manufacturer's instructions, then repeat steps 3-6 twice, and proceed to step 8.
8. 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.

Quality Control

It is advisable to run the appropriate negative controls. Negative controls establish background fluorescence and non-specific staining of the primary and secondary antibodies. The ideal negative control reagent is a mouse monoclonal or myeloma protein. It should be isotype-matched to the primary antibody, not specific for human cells and of the same concentration as the primary 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.

For fluorescence analysis of cells with Fc receptors, the use of isotype matched negative control is mandatory. In some systems, it may be necessary to incubate the cells (at step 2 before adding monoclonal antibody) in 10-20% normal serum from the second antibody host species in order to decrease non-specific staining with the conjugated second antibody.

Product Profile

When assayed by flow cytometric analysis, using 10 µl of the antibody to stain 1 x 10^6 peripheral blood 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.

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

General References:

1. Griffin, H.T., et.al., Generation of monoclonal antibodies to a human natural killer clone. Characterization to two natural killer-associated antigens, NKH1A and NKH2, expressed on subsets of large granular lymphocytes. J. Clin. Invest., 75(3), 932-943 (1985).
2. Lanier, L.L., et al., The relationship of CD16 (Leu-11) and Leu-19 (NKH-1) antigen expression on human peripheral blood NK cells and cytotoxic T lymphocytes. J. Immunol.,136(12), 4480-4486 (1986).
3. Schmidt, R.E., et al., A subset of natural killer cells in peripheral blood displays a mature T cell phenotype. J. Exp. Med., 164(1), 351-356 (1986).
4. Caligiuri, M., et. Al., Phenotypic and functional deficiency of natural killer cells in patients with chronic fatigue syndrome. J. Immunol., 139(10), 3306-3313 (1987).
5. Hercend, T., and Schmidt, R.E., Characteristics and uses of natural killer cells. Immunology Today, 9(10), 291-293 (1988).

lpg 6/01