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 HLA-DQ
CLONE HK19
FITC Conjugate
Purified Mouse Immunoglobulin

Product No. **F 1777**

Product Description

Monoclonal Anti-Human HLA-DQ (mouse IgG2b isotype) is derived from the HK19 hybridoma produced by the fusion of SP2/0-Ag14 mouse myeloma cell line and splenocytes from BALB/c mice immunized with GM-1500 human tumor cells. The isotype is determined using the Sigma ImmunoType™?Kit (Product Code ISO-1) and by a double diffusion assay using Mouse Monoclonal Antibody Isotyping Reagents (Product Code ISO-2). The product is prepared by conjugation of fluorescein isothiocyanate (FITC) Isomer I with purified HLA-DQ monoclonal antibody. The conjugate is then purified by gel filtration to remove unbound FITC; no free FITC is detectable.
Monoclonal Anti-Human HLA-DQ recognizes MHC class II molecules that are preferentially expressed on the surface of B lymphocytes. The antigen molecules are bimolecular glycoprotein complexes of 29 kDa and 33 kDa components. The HLA-DQ antigen is present on peripheral blood lymphocytes, tonsillar and splenic mononuclear cells, and other B cell markers. Monoclonal Anti-HLA-DQ reacts weakly with resting or Con A-activated cells, monocytes, granuloctes and erythrocytes.

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

F/P Molar Ratio: 7.0

Uses

Monoclonal Anti-Human HLA-DQ may be used for:
1.) Studies characterizing Leukeimias
2.) Studies of differentiation between B lymphocytes and monocytes
3.) HLA-DQ region gene expression studies

Reagents

The antibody is provided (80 µg/ml) as a solution in 0.01 M phosphate buffered saline, pH 7.4, containing 1% bovine serum albumin with 0.1% 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 hazards and safe handling practices.

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.

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

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® (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, Product No. F 6647).
4. 12 x 75 mm test tubes.
5. Adjustable micropipet.
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 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 FITC conjugated, isotype-matched non-specific mouse immunoglobulin (Product No. F 6647) 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.
   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.

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

Shipp, M., et al., J. Immnol., **131**, 2458 (1983).
Shackelford, D., et al., Proc. Natl. Acad. Sci. USA, **78**, 4566 (1981).
Shipp, M., et al., Human Immunology, **16**, 24 (1986).

12/98