SIGMA®
CHEMICAL COMPANY

Product No. F-1902
Lot 066H8815

Monoclonal Anti-Human HLA-DR
FITC Conjugate
Purified Mouse Immunoglobulin
Clone HK14

Monoclonal Anti-Human HLA-DR (mouse IgG2a isotype) is derived from the HK14 hybridoma produced by the fusion of SP2/0-Ag14 mouse myeloma cells and splenocytes from BALB/c mice immunized with the GM-1500 human tumor cell line[1]. The isotype is determined by a double diffusion assay using Mouse Monoclonal Antibody Isotyping Reagents (Sigma Stock No. ISO-2). The product is prepared by conjugation of fluorescein isothiocyanate (FITC) to purified human HLA-DR monoclonal antibody. The product is then purified by gel filtration to remove unbound FITC, no free FITC is detectable. The conjugate is provided as a solution (30 µg/ml) in 0.01 phosphate buffered saline, pH 7.4, containing 1% BSA and 0.1% sodium azide (see MSDS)* as a preservative.

Description

Monoclonal Anti-Human HLA-DR from clone HK14 is specific for a non-polymorphic HLA-DR determinant. The HLA-DR antigens are also known as MHC class II molecules and are encoded by the human major histocompatability complex. Class II molecules are composed of 33-36 kD alpha and 24-30 kD beta chains, and have the function of binding processed antigen and presenting it to antigen-specific T helper cells.[2,3] Class II molecules are expressed on antigen presenting cells such as macrophages, B cells, dendritic cells and activated T cells.[2,4,5] One report cites evidence for expression on granulocytes.[5] Under certain conditions, class II molecules may be expressed on other non-hematopoietic, non-lymphoid cells.[4]

F/P Molar Ratio: 6.6

Performance

When assayed by flow cytometric analysis, using 10 µl of the conjugate to stain 1 x 10^6 cells, a fluorescence intensity and percent positive is observed similar to that obtained with saturating monoclonal antibody levels.

Uses

FITC Monoclonal Anti-Human HLA-DR may be used for:
1. Identification of B cells, a percentage of monocytes, and activated T cells.
2. Identification of certain 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.

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® (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 conjugated, isotype-matched, non-specific mouse immunoglobulin (negative control, Sigma Product No. F-6397).

Reagents and Materials Needed but Not Supplied (cont.)

4. 12 x 75 mm test tubes.
5. Adjustable micropipet.
6. Centrifuge.
7. Counting chamber.
8. Trypan blue (Sigma 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 (Sigma Product No. F-6397) 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

1. Shipp, M., J. Immunol., **131(5)**, 2458 (1983).
2. Shackelford, D., Immunol. Rev., **66**, 133 (1982).
3. Spies, T., Proc. Nat. Acad. Sci., **82**, 5165 (1985).
4. Accolla, R., S. Sem. Hematol, **21(4)**, 287 (1984).
5. Keren, D. (ed), Flow Cytometry in Clinical Diagnosis, ASCP Press, Chicago, 84-85 (1989).

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