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Product Information

Monoclonal Anti-HLA-DR-PE, clone HK14
produced in mouse, purified immunoglobulin

Catalog Number **P8950**

Product Description

Monoclonal Anti-HLA-DR (mouse IgG2a isotype) is derived from the HK14 hybridoma produced by the fusion of SP2/0-Ag14 mouse myeloma cell line NS-1 and splenocytes from BALB/c mice immunized with GM-1500 human tumor cells.[1] The isotype is determined by a double diffusion assay using Mouse Monoclonal Antibody Isotyping Reagents, Catalog Number ISO2. The product is prepared by conjugation of R-Phycoerythrin (PE) with purified HLA-DR monoclonal antibody. The conjugate is then purified by gel filtration to remove unbound PE and antibody. No free PE or free antibody is detectable.

Monoclonal Anti-HLA-DR, clone HK14, is specific for a non-polymorphic HLA-DR derminant. 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 kDa alpha and 24-30 kDa 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]

Monoclonal Anti-HLA-DR may be used for identification of B cells, activated T cells and a percentage of monocytes and for identification of certain leukemias and lymphomas.

Reagent

Supplied as a solution (100 µg/ml) in 0.01 M phosphate buffered saline, pH 7.4, containing 1% BSA with 0.1% sodium azide as a preservative.

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Product Profile

When assayed by flow cytometric analysis, using 10 µl of the conjugate to stain 1 x 10^6 cells, a fluorescent 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.

A_{567}/A_{280}: 2.1

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®, Catalog Number 10771.
2. Diluent: 0.01M phosphate buffered saline (PBS), pH 7.4, containing 1% BSA and 0.1% NaN_3.

3. PE conjugated, isotype-matched, non-specific mouse immunoglobulin (negative control, Catalog No. P4810).
4. 12 x 75 mm test tubes.
5. Adjustable micropipet.
6. Centrifuge.
7. Counting chamber.
8. Trypan blue, Catalog No. 302643, 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 ml 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 Fl 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 PE conjugated, isotype-matched non-specific mouse immunoglobulin (Catalog No. P4810) 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.

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 that 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. Shakelford, D., Immunol. Rev., **66**, 133 (1982).
3. Spies, T., Proc. Nat. Acad. Sci., **82**, 5165 (1985).
4. Keren, D. (ed.), Flow Cytometry in Clinical Diagnosis, ASCP Press, Chicago, 84 (1989).

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