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Product Information

Monoclonal Anti-HLA-DR- Quantum Red™
Clone HK14
produced in mouse, purified immunoglobulin

Catalog Number **R8144**

Product Description

Monoclonal Anti-HLA-DR (mouse IgG2a isotype) is derived from the HK14 hybridoma produced by the fusion of mouse myeloma cells and splenocytes from BALB/c mice immunized with the GM-1500 human tumor cell line.[1] The product is prepared by conjugation of Quantum Red with purified HLA-DR monoclonal antibody. Quantum Red is Sigma's tandem fluorochrome in which a small organic dye, Cy5, is covalently linked to R-Phycoerythrin (PE). The PE absorbs light energy at 488 nm and emits in the excitation range of Cy5 that acts as the acceptor dye. The complex then emits at 670 nm. The conjugate is purified by gel filtration to remove unbound Quantum Red and antibody. No free Quantum Red 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 α and 24-30 kDa β 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-Human HLA-DR- Quantum Red may be used for:

1. Identification of B cells, activated T cells, and a percentage of monocytes.
2. Identification of certain leukemias and lymphomas.

Reagent

Supplied as a solution (40 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.

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 population positive is observed similar to that obtained with saturating monoclonal antibody levels.

A_{567}/A_{280}: 2.0

Storage/Stability

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

Procedure

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: 0.01 M phosphate buffered saline (PBS), pH 7.4, containing 1% BSA and 0.1% sodium azide.
3. Phycoerythrin-Cy5 tandem dye conjugated, isotype-matched, non-specific mouse immunoglobulin.
4. 12 x 75 mm test tubes.
5. Adjustable micropipet.
6. Centrifuge.
7. Counting chamber.

8. Trypan blue, Catalog Number 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 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.
   c. 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.
2. a. Proper controls to be included for each sample include:
   An autofluorescence control: 10 µl diluent in place of monoclonal antibody, followed by steps 3 - 7.
   A negative staining control: 10 µl of Phycoerythrin-Cy5 tandem dye conjugated, isotype-matched, non-specific mouse immunoglobulin 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., et al., J. Immunol., **131**, 2458 (1983).
2. Shakelford, D., et al., Immunol. Rev., **66**, 133 (1982).
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4. Accolla, R. S., et al., Sem. Hematol., **21**, 287 (1984).
5. Keren, D. (ed.), Flow Cytometry in Clinical Diagnosis, ASCP Press, Chicago, 84 (1989).

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