

PN IM2659**100 tests
10 µL/test****HLA-DR - PC5****(IMMU357)****IOTest[®]**
Conjugated Antibodies

For Research Use Only. Not for use in diagnostic procedures.

SPECIFICITY

The human major histocompatibility complex (MHC) constitutes a group of structurally and functionally related genes involved in the regulation of immune response. The MHC also called human leukocyte antigens (HLA) is located on the short arm of the chromosome 6. The MHC complex encodes three groups of molecules designated MHC class I, class II and class III. The MHC class I molecules present peptides derived by proteolysis of intracellular proteins (the endogenous pathway). In contrast, the MHC class II molecules bind peptides generated in the exogenous pathway (endosomal / lysosomal). In the intracellular compartment, antigens that have entered the cells via pinocytosis or via receptor-mediated internalization are processed, and antigenic peptides are bound to the HLA class II molecule (1). The HLA class III complex regroups genes from the complement cascade (C4, C2, and factor B) as well as the tumor necrosis factors (TNF) genes (2).

The HLA class II region, also called HLA-D region, contains the genes encoding HLA-DR, -DQ and -DP antigens (1,2). The HLA class II molecules like class I molecules, are composed of non-covalently associated alpha / beta heterodimers. On HLA class II, both heavy (alpha) and light (beta) chain with a molecular weight of 31-33 kDa and 26-29 kDa respectively span the cell membrane (1). The two immunoglobulin-like domains proximal to the cell membrane (alpha 2 and beta 2) support the two polymorphic amino-terminal domains (alpha 1 and beta 1) distal to the membrane which constitute a part of the "antigen-presenting site" (2). The HLA class II is involved in the presentation of peptide fragments to the restricted CD4+ T lymphocyte subpopulation (T helper / inducer) resulting than in an enhancement of the immune response (1,3). Intercellular communication events which drive this response are mediated by the MHC presenting the antigen (Ag), the T cell receptor (TcR) with the CD4 molecule recognizing the complex MHC-Ag, and accessory molecules intensifying the cellular interaction (3,4,5). Unlike the HLA class I molecules that are expressed on virtually all somatic cells, the HLA-D (including the DR haplotype) is found on limited cell populations known as "antigen presenting cells" (APC: i.e. B-lymphocytes, monocytes / macrophages, dendritic cells, Langerhans cells of the skin) (2,6). On T-lymphocytes the HLA-DR is only expressed after activation (7). The HLA-DR is also expressed on some hematopoietic progenitor cells at different stages of differentiation (2,8). IMMU357 monoclonal antibody recognizes a monomorphic HLA-DR epitope with a molecular weight of 29-33 kDa.

REAGENT

Clone IMMU357
Isotype IgG1 mouse
Immunogen EBV-transformed cell line
Hybridoma P3-X63-Ag 8 653 x Balb/c spleen cells
Source Ascites fluid
Purification Ion exchange or affinity chromatography
Conjugation PC5 The IgG is conjugated to a tandem dye constituted of R-phycoerythrin covalently linked to cyanin 5.1 at 0.7-1 mole of PC5 per mole of IgG
Excitation wavelength: 488 nm
Maximum emission wavelength 670 nm
Main emission color: Deep-red
Buffer 2 mg/mL bovine serum albumin in phosphate-buffered saline containing 0.1% sodium azide

APPLICATION

Flow cytometry.

Enumeration and characterization of cell subsets expressing the HLA-DR antigen

Studies of the involvement of HLA Class II (DR haplotype) antigens in cellular interactions and antigenic stimulation

Studies of cell-surface expression of HLA Class II antigens during nematopoptosis.

STATEMENT OF WARNINGS

1. This reagent contains 0.1% sodium azide. Sodium azide under acid conditions yields hydrazoic acid, an extremely toxic compound. Azide compounds should be flushed with running water while being discarded. These precautions are recommended to avoid deposits in metal piping in which explosive conditions can develop. If skin or eye contact occurs, wash excessively with water.
2. Specimens, samples and all material coming in contact with them should be handled as if capable of transmitting infection and disposed of with proper precautions.
3. Never pipet by mouth and avoid contact of samples with skin and mucous membranes.
4. Do not use antibody beyond the expiration date on the label.
5. Do not expose reagents to strong light during storage or incubation.
6. Avoid microbial contamination of reagents or incorrect results might occur.

STORAGE CONDITIONS AND STABILITY

Each reagent is stable up to the expiration date when stored at 2-8 C. Do not freeze. Minimize exposure to light.

REAGENT PREPARATION

No reconstitution is necessary. This monoclonal antibody may be used directly from the vial. Bring reagent to 20 - 25 C prior to use.

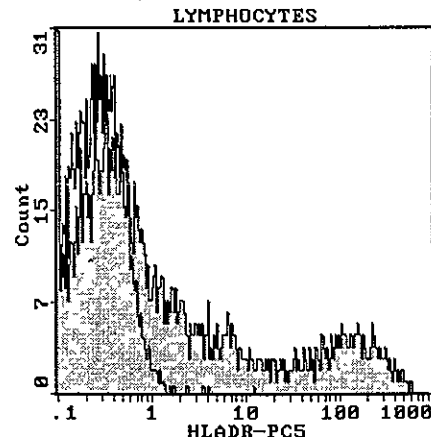
PROCEDURE

This reagent is designed for Flow Cytometry
 Assay volume: 10 µL/5 x 10⁵ cells / test or 100µL whole blood.
 A wash is required to yield optimal results.

EXAMPLE DATA

The histograms below are monoparametric representations (Count versus Fluorescence Intensity) of lysed normal whole blood sample. Staining is with HLA-DR-PC5 monoclonal antibody (PN IM2659) gated on lymphocytes. The isotopic control labeling (PN IM2663) is underneath in light.

Acquisition is with a COULTER R EPICS R XL TM cytometer
 Analysis is with the XL System II TM software.



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COULTER

PARTNERS IN CELL ANALYSIS

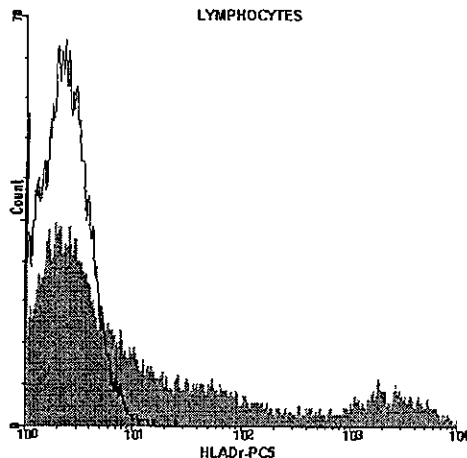
 **IMMUNOTECH**
 A COULTER COMPANY

PN IM2659 HLA-DR - PC5 (IMMU357)

100 tests
10 µL/test

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Acquisition is with a Becton Dickinson FACScan TM flow cytometer.
Analysis is with the LYSYS II TM software.



SELECTED RESEARCH REFERENCES

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- 3-[2831] Wade, W.F., Davoust, J., Salamero, J., André, P., Watts, T H , Cambier, J C , "Structural compartmentalization of MHC class II signaling function", 1993, *Immunol Today*, 11, 14, 539-546
- 4-[2354] Schick, M R., Levy, S , "The TAPA-1 molecule is associated on the surface of B cells with HLA-DR molecules", 1993, *J. Immunol.*, 8, 151, 4090-4097.
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- 8-[3487] Huang, S , Terstappen, L W M.M., "Lymphoid and myeloid differentiation of single human CD34+, HLA-DR, CD38-hematopoietic stem cells", 1994, *Blood*, 6, 83, 1515-1526.

