

PN IM3635**100 tests
10 µL / test****HLA-DR-APC****(Immu-357)****IO Test®**

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 leucocyte 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 α/β heterodimers. On HLA class II, both heavy (α) and light (β) 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 - 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).

Immu-357 monoclonal antibody recognizes a monomorphic HLA-DR epitope with a molecular weight of 29 - 33 kDa.

REAGENT

Clone Immu-357
Isotype IgG1, mouse
Immunogen EBV-transformed cell line
Hybridoma P3-X63-Ag.8.653 x Balb/c
Source Ascites fluid
Purification Ion exchange or affinity chromatography
Conjugation Allophycocyanin (APC) is conjugated at 0.5 - 1.5 moles of APC per mole of Ig.
 Excitation wavelength: 633 - 635 nm
 Maximum emission wavelength: 660 nm
 Main emission color: Deep-red
 Limitation: APC conjugates are recommended for use only on flow cytometers equipped with an exciting source of 633 nm (He-Ne laser) or 635 nm (Red diode laser).

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 hematopoiesis.

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 18 - 25°C prior to use.

PROCEDURE

This reagent is designed for Flow Cytometry.

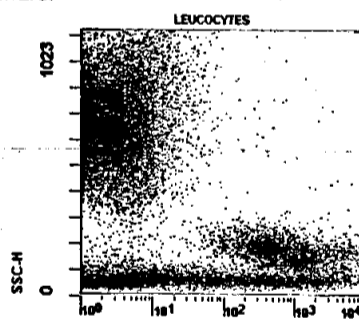
Assay volume: 10 µL per 5×10^5 cells in one test, or per 100 µL whole blood.

A wash is required to yield optimal results.

EXAMPLE DATA

The histogram below is a biparametric representation (Side Scatter versus Fluorescence Intensity) of a lyzed normal whole blood sample. All leucocytes are shown. Staining is with HLA-DR-APC monoclonal antibody (PN IM3635). Isotypic control labelling (PN IM2475) is not shown.

Acquisition is with a BD Biosciences FACSCalibur™ flow cytometer equipped with CELLQuest™ software. Analysis is with EXPO 32™ Cytometer software.



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**COULTER**

PARTNERS IN CELL ANALYSIS

**IMMUNOTECH**
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SELECTED RESEARCH REFERENCES

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3. 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. 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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3635EX100901 Vers. 01/ 26/10/01 AC-01-1652

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