

PN IM3636**HLA-DR-ECD****(Immu-357)**
IO Test[®]
 Conjugated Antibodies

100 tests
10 µL/test
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 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.8653 x Balb/c spleen cell
Source	Ascites fluid
Purification	Ion exchange or affinity chromatography
Conjugation	R-phycoerythrin covalently linked to Texas Red (PE-TxR or ECD) is conjugated at 0.5 - 1.5 moles of ECD per mole of Ig. Excitation wavelength: 488 nm Maximum emission wavelength: 613 nm Main emission color: 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 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 extensively 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.

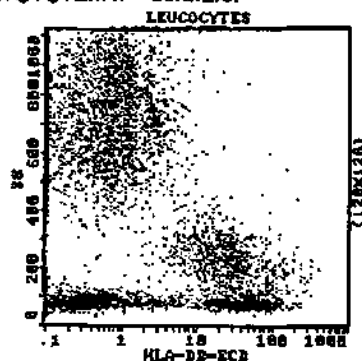
 Assay volume: 10 µL per 5 x 10⁶ cells in one test or per 100 µL whole blood.

 This reagent works with either a wash or a no-wash procedure, such as COULTER[®] TQ-Prep[™] Workstation with ImmunoPrep[™] Reagent (PN 7546999) System.

EXAMPLE DATA

The histogram below is a biparametric representation (Side Scatter versus Fluorescence Intensity) of a lysed normal whole blood sample using the no-wash procedure. All leucocytes are shown. Staining is with HLA-DR-ECD monoclonal antibody (PN IM3636). Isotypic control labeling (PN IM2714) is not shown.

Acquisition is with a COULTER[®] EPICS[®] XL[™] flow cytometer. Analysis is with SYSTEM II[™] software.



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PARTNERS IN CELL ANALYSIS


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8. Huang, S., Terstappen, L.W.M.M., "Lymphoid and myeloid differentiation of single human CD34⁺, HLA-DR⁺, CD36⁺ hematopoietic stem cells", 1994, *Blood*, 8, 83, 1515-1526.

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