

PN IM2053**50 tests
20 µL/test****CD3 - FITC
HLA-DR - PE****IO Test[®]**
Conjugated Antibodies

For Research Use Only Not for use in diagnostic procedures.

SPECIFICITY**CD3**

T lymphocytes constitute the majority of human peripheral blood lymphocytes (PBL) (1) T lymphocytes are characterized by the expression the T cell receptor (TcR) associated with the CD3 antigen and are commonly divided into two subsets. "helper / inducer" (or Th) lymphocytes expressing CD4 antigen and "suppressor / cytotoxic" (or Tc) lymphocytes expressing the CD8 molecule (1,2)

The CD3 antigen is a complex of 5 polypeptide chains α , β , ϵ , ζ and η associated with the TcR These chains are composed by a group of two invariant dimers, γ - ϵ and δ - ϵ associated with a variable dimer (3)

The CD3 complex associated with the TcR is involved in the recognition of peptides bound to the major histocompatibility complex I and II (MHC) during the immune response (4)

The CD3 antigen is expressed by mature T lymphocytes and by a subset of thymocytes (5) In human PBL, approximately 67 to 76% of the lymphocytes are CD3+ The range of percentage is lower during childhood and may vary with aging (1)

The UCHT1 monoclonal antibody reacts with the ϵ chain of the CD3 complex (6)

The UCHT1 monoclonal antibody has been assigned to the CD3 cluster of differentiation at the 1st International Workshop on Human Leucocyte Differentiation Antigens in Paris (1982) (7) HLA-DR

The major histocompatibility complex (MHC) constitutes a group of structurally and functionally related genes involved in the regulation of immune response On human the MHC also called human leucocyte antigens (HLA) is located on the short arm of the chromosome 6 The MHC antigens are involved in the presentation of antigenic peptides to the immune system (8). The MHC complex encodes three groups of molecules designated MHC class I, class II and class III The MHC class II molecules bind peptides generated in the exogenous pathway (endosomal / lysosomal) (9)

The HLA class II region, also called HLA-D region, contains the genes encoding HLA-DR, -DQ and -DP antigens (8,9) 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 (8)

The HLA class II is involved in the presentation of peptide fragments to the restricted CD4+ T lymphocyte subpopulation (T helper / inducer) resulting in an enhancement of the immune response (8,10) 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) (9,11). On T-lymphocytes the HLA-DR is only expressed after activation (12) The HLA-DR is also expressed on some hematopoietic progenitor cells at different stages of differentiation (9,13)

IMMU357 monoclonal antibody recognizes a monomorphic HLA-DR epitope with a molecular weight of 29-33 kDa

REAGENT

CD3	HLA-DR
UCHT1	IMMU357
IgG1 mouse	IgG1 mouse
NS1 x Balb/c spleen cells	P3-X63-Ag 8 653 x Balb/c spleen cells

Source Ascites fluid**Purification** Ion exchange or affinity chromatography**Conjugations** FITC Fluorescein isothiocyanate (FITC) is conjugated at 3 - 6 moles of FITC per mole of IgG

Excitation wavelength 488 nm

Maximum emission wavelength 525 nm

Main emission color Green

PE R-phycoerythrin (PE) is conjugated at 0.7-1 mole of PE per mole of IgG

Excitation wavelength 488 nm

Maximum emission wavelength 575 nm

Main emission color Orange-red

Buffer 2 mg/mL bovine serum albumin in phosphate-buffered saline containing 0.1% sodium azide**APPLICATION**

Flow cytometry

Characterization of activated T lymphocytes (CD3+ HLA-DR+)

Characterization of a differential expression of HLA-DR antigens on CD3+ and / or CD3- lymphocyte subsets 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 20 - 25 °C prior to use

PROCEDURE

This reagent is designed for Flow Cytometry

Assay volume 20 µL/5 x 10⁵ cells / test or 100µL whole blood

A wash is required to yield optimal results

EXAMPLE DATA

The graphs below are biparametric representations (Fluorescence Intensity versus Fluorescence Intensity) of a lyzed normal whole blood sample Staining is with CD3-FITC / HLA-DR-PE dual color reagent (PN IM2053) gated on lymphocytes

*Upper left quadrant (1) contains CD3- HLA-DR+ lymphocytes

*Upper right quadrant (2) contains CD3+ HLA-DR+ lymphocytes representing activated T lymphocytes

*Lower left quadrant (3) contains double negative lymphocytes CD3- HLA-DR-

*Lower right quadrant (4) contains non activated T lymphocytes CD3+ HLA-DR-

**COULTER**

PARTNERS IN CELL ANALYSIS

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**IMMUNOTECH**
A COULTER COMPANY

PN IM2053

**50 tests
20 µL/test**

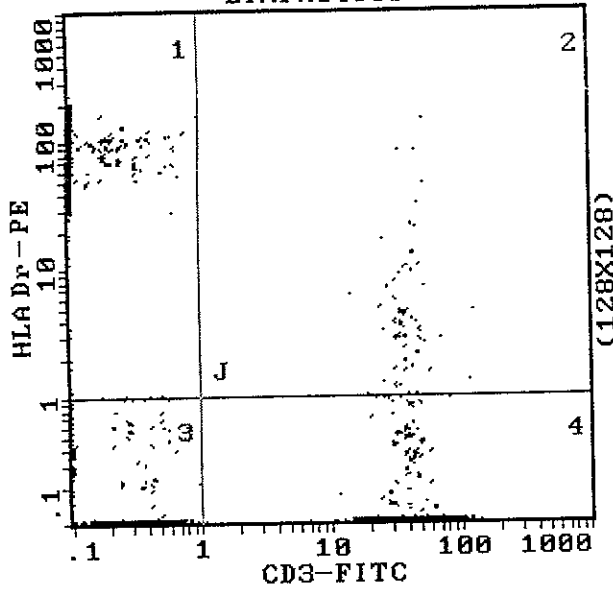
CD3 - FITC

HLA-DR - PE

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

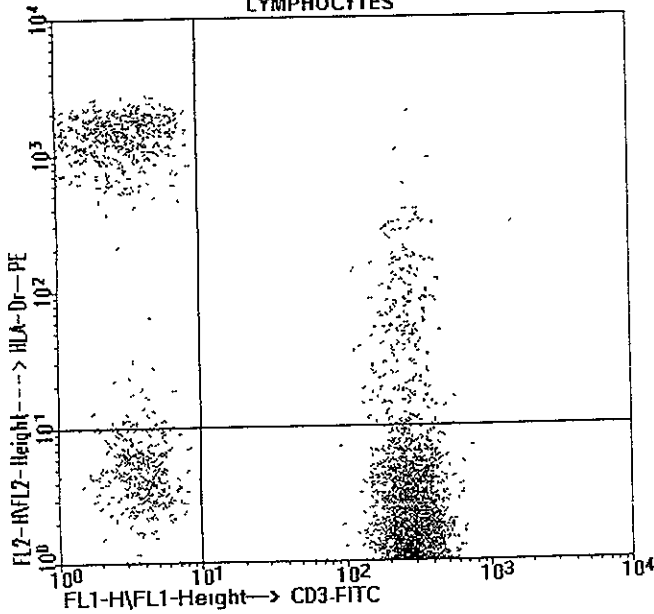
LYMPHOCYTES



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

LYMPHOCYTES



SELECTED RESEARCH REFERENCES

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