IOTest® HLA-DR-FITC / CD8-PE

PN IM1199U – 1 mL Liquid – 20 µL / test*

Analyte Specific Reagent.

Analytical and performance characteristics are not established.

REAGENT COMPONENTS

	Specifications of constituent 1	Specifications of constituent 2
Specificity	HLA-DR	CD8
Clone	B8.12.2	B9.11
Hybridoma	NS1 x Balb/c	NS1 x Balb/c
Immunogen	Human HLA A6 cytotoxic T-cell clone	Human HLA A2 cytotoxic T-cell clone
Ig Chain	IgG2b	IgG1
Species	Mouse	Mouse
Source	Ascites fluid	Ascites fluid
Purification	Ion exchange or affinity chromatography	Ion exchange or affinity chromatography
Conjugation	FITC (Fluorescein isothiocyanate)	PE (Phycoerythrin)
Molar Ratio	FITC / Ig: 5 – 7	PE / Ig: 0.5 – 1.5
λ Excitation range	468 – 509 nm	486 – 580 nm
λEmission range	504 – 541 nm (green)	568 – 590 nm (Orange)
Buffer	Buffer (PBS pH 7.2) plus 2 mg / mL BSA and 0.1% NaN_3	

SPECIFICITY

The human major histocompatibility complex (MHC), also called human leucocyte antigens (HLA), is composed of three groups of molecules designated MHC class I, class II and class III. The MHC class II genomic region, or HLA-D region, contains the genes encoding HLA-DR, -DQ and -DP antigens (1, 2). The MHC class II molecules are built by the non-covalent association of α/β heterodimers. Both heavy (α) and light (β) chains span the cell membrane (1). They have molecular weights of 31 – 33 kDa and 26 – 29 kDa respectively. The HLA-DR antigen is found on B-lymphocytes, monocytes / macrophages, dendritic cells, and Langerhans cells (2, 3). On T-lymphocytes, the HLA-DR is only expressed after activation. HLA-DR is also expressed on some hematopoietic progenitor cells at different stages of differentiation (2, 4).

The B8.12.2 monoclonal antibody (mAb) recognizes a monomorphic HLA-DR epitope with a molecular weight of 29-33 kDa (5).

The CD8 molecule is a disulfide-linked dimer composed by an α- and a β-chain. Both α and β subunits have a molecular weight (Mr) of 32 – 34 kDa (6). The CD8 antigen is expressed by the "cytotoxic / suppressor" T lympocytes subpopulation (Tc cells) and with a lower density by a subset of NK cells (7). The majority of Tc cells express the CD8 molecule as an α/β heterodimer whereas NK cells are essentially $CD8\alpha^+/\beta^-$ (or $CD8\alpha^+/\alpha^+$) (7, 8).

The B9.11 mAb reacts with the α subunit of the CD8 molecule (6). It has been assigned to the CD8 cluster of differentiation during the 1st International Workshop on Human Leucocyte Differentiation Antigens in Paris, France, in 1982 (9).

REAGENT

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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. Do not use reagent beyond the expiration date on the vial label.
3. All specimens and samples must be considered as potentially infectious and must be handled with care (in particular: the wearing of protective gloves, gowns and goggles).
4. Do not expose reagents to strong light during storage or incubation.
5. Avoid microbial contamination of reagents or incorrect results might occur.
6. Avoid contact of samples with skin mucosa and eyes. Never pipet by mouth
7. Let it come to room temperature (18 – 25°C) before use.
8. Use general good laboratory practices when handling this reagent.

STORAGE CONDITIONS AND STABILITY

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

EVIDENCE OF DETERIORATION

Any change in the physical appearance of this FITC- and PE-labeled combination (clear, yellowish-green to pinkish liquid) or any major variation in values obtained for control samples may indicate deterioration and the reagent should not be used.

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.

SELECTED RESEARCH REFERENCES

1. Krensky, A.M., "The HLA system, antigen processing and presentation", 1997, Kidney International, suppl. 58, 51, 2-7.
2. Lee, J., Dupont, B.O., The HLA system : an introduction", 1990, *in*: "The HLA system: A new approach", Springer-Verlag, 1-26.
3. Uckun, F.M., "Regulation of human B-cell ontogeny", 1990, Blood,10, 76, 1908-1923.
4. 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.
5. Rebai, N., Malissen, B., Pierres, M., Acolla, R.S., Corte, G., Mawas, C., "Distinct HLA-DR epitopes and distinct families of HLA-DR molecules defined by 15 monoclonal antibodies (mAb) either anti-DR or allo-anti-Iak cross-reacting with human DR molecule I. Cross-inhibition studies of mAb cell surface fixation and differential binding of mAb to detergent-solubilized HLA molecules immobilized to a solid phase by a first mAb", 1983, Eur. J. Immunol., 13, 106-111.

(*): 20 µL is the quantity of product sufficient to stain 5×10^5 cells in a standard immunofluorescence assay

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6. Alcover, A., "CD8 cluster report", 1995, Leucocyte Typing V, White Cell Differentiation Antigens. Schlossman, S.F., et al., Eds., Oxford University Press, 353-354.
7. Terry, L.A., DiSanto, J.P., Small, T.N., Flomenberg, N., "Differential expression and regulation of the human CD8α and CD8β chains", 1990, Tissue Antigens, 35, 82-91.
8. Baume, D.M., Caligiuri, M.A., Manley, T.J., Daley, J.F., Ritz, J., "Differential expression of CD8α and CD8β associated with MHC-restricted and non-MHC-restricted cytolytic effector cells", 1990, Cell. Immunol., 131, 352-365.
9. Bernard, A., Brottier, P., Georget, E., Lepage, V., Boumsell, L., "Joint report of the first international workshop on human leucocyte differentiation antigens by the investigators of the participating laboratories", 1984, Leucocyte Typing I, Bernard, A. et al., Eds., Springer Verlag, 9-135.

PRODUCT AVAILABILITY

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PE is licensed under patent 4,520,110.

For additional information in the USA, call 800-526-7694.
Outside the USA, contact your local Beckman Coulter representative.

www.beckmancoulter.com

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