

PRODUCT INFORMATION SHEET

Monoclonal antibodies detecting human antigens

CD45RO FITC **CD4** R-PE RUO REF IQP-267FR 50 tests

RUO *For Research Use Only*



Description

The combination of CD4 and CD45RO can be used to detect memory CD4 positive T cells among the peripheral blood lymphocytes. These cells are further characterized by, for instance, the lack of expression of CD27. Naive CD4 positive T cells have not been activated yet and express CD45RA and CD27.

CD45RO

Clone UCHL1

Isotype Murine IgG2a

Specificity Clone UCHL1 recognizes memory and activated T cells in peripheral blood, and T cell tumors, and results from the activation of the CD45RA isoforms. UCHL1 reacts with lymphocytes in T cell areas of normal lymphoid tissues. Monocytes and macrophages may also show strong expression of CD45RO.

HLDA Workshop

3rd International Workshop on Human Leukocyte Differentiation Antigens.

CD4

Clone Edu-2

Isotype Murine IgG2b

Specificity Monoclonal antibodies clustered as CD4 detect most thymocytes and a subpopulation of peripheral blood T cells, called T helper cells (Th). In addition, CD4 is expressed on monocytes and macrophages. The CD4 antigen is a 55 kD glycoprotein which plays a role in the recognition of foreign antigens presented to T cells by MHC class II molecules. Furthermore, CD4 acts as a receptor for HIV-1 by binding the viral protein gp120.

HLDA Workshop

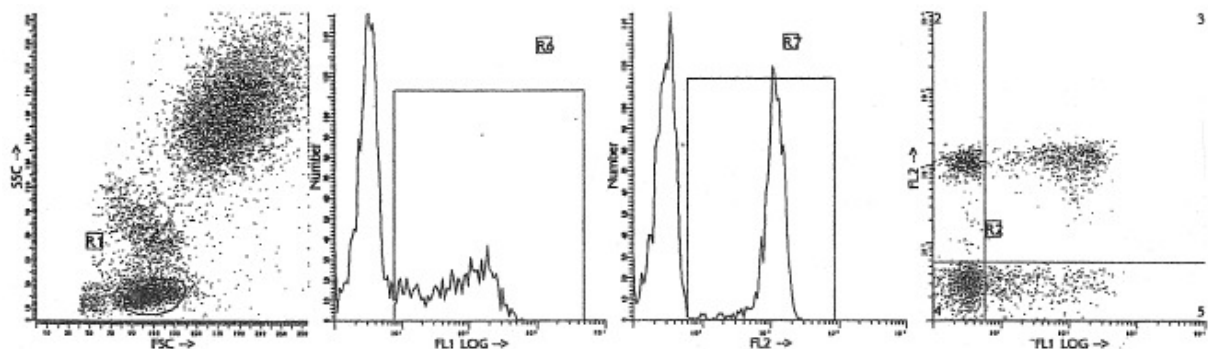
5th International Workshop on Human Leukocyte Differentiation Antigens

Usage

All these reagents are effectively formulated for direct immunofluorescent staining of human tissue for flow cytometric analysis using 10 µl/10⁶ leukocytes for singles and 20 µl/10⁶ leukocytes in case of dual and triple combinations. Since applications vary, each investigator should titrate the reagent to obtain optimal results.

Representative Data

Staining with the dual CD45RO FITC and CD4 R-PE was performed using 20 µl of the conjugated monoclonal antibody preparation and 100 µl of blood sample.



Immunofluorescence staining and lysing protocol

Flow cytometry method for use with dual and triple combinations

1. Add 100 µl of EDTA-treated blood (i.e. approx. 10^6 leukocytes) to a 5 ml reagent tube. The content of one tube is sufficient to perform one test.
For combinations with anti-kappa and/or anti-lambda Ig see application note below.
2. Add to each tube 20 µl of labeled monoclonal antibody combination*.
3. Vortex the tube to ensure thorough mixing of antibody and cells.
4. Incubate the tube for 15 minutes at room temperature in the dark.
5. Add 100 µl of IQ Lyse (IQP-199 ready-to-use) and mix immediately.
6. Incubate for 10 minutes at room temperature in the dark.
7. Add 2 ml of demineralized water and incubate for 10 minutes in the dark.
8. Centrifuge the labeled cell suspension for 2 minutes at 1000 x g.
9. Remove the supernatant and resuspend the cells in 200 µl of PBS**.
10. Analyze by flow cytometry within four hours (alternatively, the cells may be fixed by 0.05% of formaline in buffered saline for analysis the next day. Some antigens are readily destroyed upon fixation and this should be taken into account when using this alternative).

* Appropriate mouse Ig isotype control samples can be included in any labeling study

** PBS: Phosphate Buffered Saline, pH 7.2

Application note for anti-kappa and/or anti-lambda Ig combinations

Add 2 ml of PBS containing 0.001% (v/v) Heparin (**prewarmed to 37 °C**) to the cell suspension
Vortex, centrifuge (2 min at 300x g) and discard the supernatant
Repeat this step twice
Resuspend the pelleted blood cells in 100 µl PBS containing 0.001% (v/v) Heparin



Handling and Storage

Antibodies are supplied either as 100 tests per vial (1 ml) for singles or 50 tests per vial (1 ml) for dual and triple combinations. They are supplied in 0.01 M sodium phosphate, 0.15 M NaCl; pH 7.3, 0.2% BSA, 0.09% sodiumazide (NaN_3). Store the vials at 2-8 °C. Monoclonal antibodies should be protected from prolonged exposure to light. Reagents are stable for the period shown on the vial label when stored properly.











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Characterization

To ensure consistently high-quality reagents, each batch of monoclonal antibody is tested for conformance with characteristics of a standard reagent. Representative flow cytometric data is included in this data sheet.

Warning All products contain sodiumazide. This chemical is poisonous and hazardous. Handling should be done by trained staff only.


Explanation of used symbols

	Consult instructions for use
	Catalogue number
	Sufficient for
	In Vitro Diagnostic medical device
	Caution, consult accompanying document
	Keep away from (sun)light
	Biological risks
	Temperature limitation (°C)
	For Research Use Only
	Batch code
	Use by yyyy-mm-dd
	Manufacturer
	Authorized Representative in the European Community
	Conformité Européenne (European Conformity)

		Label - tandem	Ex -max (nm)	Em -max (nm)
P	PURE	purified material	-	-
F	FITC	FITC	488	519
R	R-PE	PE	488, 532	578
C	CyQ	PE-Cy5.18	488, 532	667
A	APC		595, 633, 635, 647	660
PC	PerCP		488, 532	678
PCC	PerCP-Cy5.5		488, 532	695



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