PRODUCT INFORMATION SHEET

Monoclonal antibodies detecting human antigens

CD3 FITC **CD8** R-PE **CD45** CyQ RUO REF IQP-418FRC 50 tests

RUO ***For Research Use Only***

Description

CD3

Clone UCHT1
For detailed description of this particular single reagent, please refer to IQP-519, CD3 (UCHT1)

Isotype Murine IgG1

Specificity Clone UCHT1 produces mouse IgG1 immunoglobulins directed against an epitope expressed on the epsilon chain of the CD3/TcR complex (22-28 kD).

CD3 antibodies are used, in the characterization of various subtypes of chronic lymphoid leukemias. Examples of these chronic T cell leukemias are T-CLL (Sézary Syndrome) and the peripheral T cell lymphoma (ATLL) which co-express CD3, CD2, CD4 and CD5 antigens. The NK cell lymphoma or the intestinal T cell lymphoma, co-express CD3, CD2 and CD8.

HLDA Workshop
6th International Workshop on Human Leukocyte Differentiation Antigens

CD8

For detailed description of this particular single reagent, please refer to IQP-104, CD8 (MCD8)

Clone MCD8

Isotype Murine IgG1

Specificity Clone MCD8 produces mouse IgG1 immunoglobulins directed against human CD8, and has been clustered as CD8 during the 6th Leukocyte Typing Workshop. The CD8 molecule is expressed as a heterodimer of CD8a (32-34 kD) and CD8b (32-34 kD) glycoproteins.
CD8 positive T cells, known as cytotoxic/suppressor cells (Tcyt), may be further sub-divided into Tc1 and Tc2 cells based on cytokine profile and functional activity. CD8 antibodies may help identify T cell leukemias, such as common T-ALL or mature T-ALL in combination with other markers such as TdT and cyCD3. It is also used to distinguish between chronic B and T cell lymphoid leukemias.

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CD45

For detailed description of this particular single reagent, please refer to IQP-124, CD45 (ML2)

Clone ML2

Isotype Murine IgG1

Specificity The CD45 molecule is also known as the leukocyte common antigen (LCA) or T200 antigen, and is comprised of different glycoproteins ranging from 180-240 kD. Expression of CD45 is found on all hemopoietic cells, e.g. granulocytes, monocytes, macrophages and lymphocytes, except mature erythroid cells. In humans, there is heterogeneous expression of CD45 isoforms on lymphocyte subpopulations, such as T cells or B cells. Monoclonal antibody ML2 has been clustered as CD45 and recognizes all forms of CD45.

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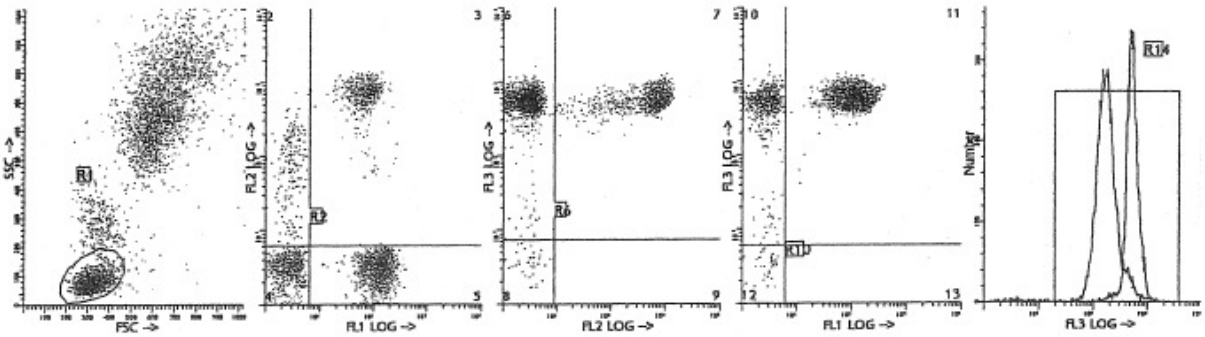
Representative Data

Staining with the triple combination of CD3 FITC, CD8 R-PE and CD45 CyQ analysis was performed using 20 µl of the conjugated monoclonal antibody and 100 µl of a washed blood sample.



Limitations

1. Conjugates with brighter fluorochromes, like PE and APC, will have a greater separation than those with dyes like FITC and CyQ. When populations overlap, the percentage of positive cells using a selected marker can be affected by the choice of fluorescent label
2. Use of monoclonal antibodies in patient treatment can interfere with antigen target recognition by this reagent. This should be taken into account when samples are analyzed from patients treated in this fashion. IQ Products has not characterized the effect of the presence of therapeutic antibodies on the performance of this reagent
3. Reagents can be used in different combinations, therefore laboratories need to become familiar performance characteristics of each antibody in relation with the combined markers in normal and abnormal samples
4. Reagent data performance is based on EDTA-treated blood. Reagent performance can be affected by the use of other anticoagulants

Reagents and materials required but not supplied

1. Flow cytometer
2. Flow cytometry disposable 12 x 75-mm capped polystyrene test tubes
3. Micropipette with disposable tips
4. Vortex mixer
5. Centrifuge
6. IQ Lyse - erythrocyte lysing solution (IQP-199)
7. IQ Starfiqs - fixation and permeabilization solution (IQP-200)
8. PBS (phosphate-buffered saline)
9. 1% Heparin
10. 1% paraformaldehyde solution in PBS (store at 2-8 °C in amber glass for up to 1 week)

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 should always 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.

Warranty Products sold hereunder are warranted only to conform to the quantity and contents stated on the label at the time of delivery to the customer. There are no warranties, expressed or implied, which extend beyond the description on the label of the product. IQ Products is not liable for property damage, personal injury, or economic loss caused by the product.

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
REF	Catalogue number
	Sufficient for
IVD	In Vitro Diagnostic medical device
	Caution, consult accompanying document
	Keep away from (sun)light
	Biological risks
	Temperature limitation (°C)
RUO	For Research Use Only
LOT	Batch code
	Use by yyyy-mm-dd
	Manufacturer
EC REP	Authorized Representative in the European Community
CE	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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