PRODUCT INFORMATION SHEET

Monoclonal antibodies detecting human antigens

CD45RC

PURE	RUO	REF	IQP-117P	100 tests	REF	IQP-117P50	50 tests
FITC	RUO	REF	IQP-117F	100 tests	REF	IQP-117F50	50 tests

RUO ***For Research Use Only***

Description

Clone MT2

Isotype Murine IgG1

Specificity Clone MT2 produces mouse IgG1 immunoglobulins reactive with a 95-115 kD highly sialated glycoprotein.

Antigen distribution

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 [1,2]. Expression of CD45 is found on all hemopoietic cells, e.g. granulocytes, monocytes, macrophages and lymphocytes, except mature erythroid cells. Detection of the different isoforms can distinguish, for example, between naive T cells and memory T cells, which is of interest in patients with immunodeficiency and autoimmune diseases.

Characteristics of the CD45R antigens

- **CD45RA (Clone MB1: IgG1)**
  Clone MB1 recognizes normal and neoplastic B cells but not mature plasma cells; monocytes, granulocytes and 50% of mature T cells [4]. MB1 does not react with non-lymphoid cell types [4]. MB1 reacts with lymphocytes in B cell areas of normal lymphoid tissues [4,5]. Naive peripheral T cells express CD45RA and not CD45RO [6].

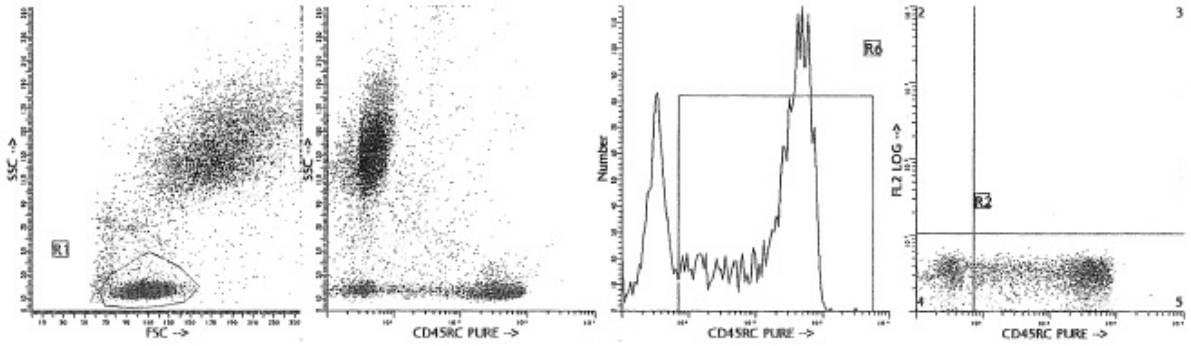
- **CD45RB (clone MT4: IgG1)**
  Variations in CD45RB expression can discriminate between T_h1 and T_h 2 cells, i.e. CD45RB-bright and CD45RB-dim respectively [8]. MT4 reacts with the 190, 205 and 220 kD isoforms of the cell-surface antigen CD45RB. CD45RB bright expression on T cells correlates with higher proliferation and IFN-g production in comparison to CD45RB dim expression. 90% of lymphocytes are CD45RB positive.

- **CD45RO (Clone UCHL1: IgG2a)**
  Clone UCHL1 recognizes memory and activated T cells in peripheral blood [6], and T cell tumors, and results from the activation of the CD45RA isoforms [6]. UCHL1 reacts with lymphocytes in T cell areas of normal lymphoid tissues [3]. Monocytes and macrophages may also show strong expression of CD45RO.

- **CD45RC (clone MT2: IgG1)**
  Clone MT2 has previously been described as CD45RA but due to its reactivity with transfectants and its identical staining pattern with ORTH75E4 it is now recognized as CD45RC [7]. Clone MT2 reacts with peripheral blood B cells and is used for the differential diagnosis of non-Hodgkin lymphomas. It also reacts with T suppressor/cytotoxic cells and NK cells.
  Their functional activity may be related to the induction of suppressor or cytotoxic activity, and activation processes.

Representative data

Flow cytometry analysis of CD45RC monoclonal antibodies is illustrated in the following cytogram. Indirect staining was performed by adding 10 µl unlabeled monoclonal antibody to 100 µl blood sample, followed by FITC-labeled secondary antibody.



Applications

The monoclonal antibodies CD45RA (clone MB1), CD45RB (clone MT4), CD45RO (clone UCHL1), and **CD45RC (clone MT2)** can be used in flow cytometry or in immunohistochemistry using cytospots, or frozen or paraffin-embedded tissue sections. Monoclonal antibodies detecting all isoforms of CD45, e.g. clone MT2, have been clustered as CD45. Other monoclonal antibodies detect the restricted epitopes (CD45R), i.e. the CD45RO, CD45RA, CD45RB and CD45RC isoforms of the CD45 complex. This restricted expression is correlated with function of the molecules and shows different expression between different subtypes of lymphoid cells.

Usage

All these reagents are effectively formulated for direct immunofluorescent staining of human tissue for flow cytometric analysis using 10 µl/10^6 leukocytes for singles and 20 µl/10^6 leukocytes in case of dual and triple combinations. Since applications vary, each investigator should titrate the reagent to obtain optimal results.

HLDA Workshop

6th Leukocyte Typing Workshop - Kishimoto er al., eds, Oxford University Press (1998).

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% paraformaldehyde solution in PBS (store at 2-8 °C in amber glass for up to 1 week)

Immunofluorescence staining and lysing protocol

- A - Flow cytometry method for use with purified monoclonal antibodies

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.
2. Add to each tube 10 µl of purified monoclonal antibody*. Vortex the tube to ensure thorough mixing of antibody and cells.
3. Incubate the tube for 15 minutes at room temperature in the dark.
4. Wash the labeled cells by adding 2 ml of PBS containing 0.001% ($^v/_v$) Heparin, vortexing and centrifuging (2 min 1000 x g.) and discard the supernatant.
5. Add 50 µl of 1:10 dilution of IQ Products $F(ab)_2$ Rabbit Anti Mouse IgG fluorescent conjugate, [FITC (IQP-190F); R-PE (IQP-190R)] in PBS containing 0.001% ($^v/_v$) Heparin to the tube. It is recommended that the tube is protected from light.
6. Mix by vortexing and incubate for 15 minutes at room temperature in the dark.
7. Add 100 µl of IQ Lyse (IQP-199 ready-to-use) and mix immediately.
8. Incubate for 10 minutes at room temperature in the dark.
9. Add 2 ml of demineralized water and incubate for 10 minutes in the dark.
10. Centrifuge the labeled cell suspension for 2 minutes at 1000 x g.
11. Remove the supernatant and resuspend the cells in 200 µl of PBS**.
12. 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).

- B - Flow cytometry method for use with labeled (FITC, R-PE, Cy-Q or APC) monoclonal antibodies

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.
2. Add to each tube 10 µl of labeled monoclonal antibody*. Vortex the tube to ensure thorough mixing of antibody and cells.
3. Incubate the tube for 15 minutes at room temperature in the dark.
4. Add 100 µl of IQ Lyse (IQP-199 ready-to-use) and mix immediately.
5. Incubate for 10 minutes at room temperature in the dark.
6. Add 2 ml of demineralized water and incubate for 10 minutes in the dark.
7. Centrifuge the labeled cell suspension for 2 minutes at 1000 x g.
8. Remove the supernatant and resuspend the cells in 200 µl of PBS**.
9. 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).

- C - 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 shoul*d 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) resp. 50 tests per vial (0.5 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.

References

1. Thomas, M.L., 1989. Annu. Rev. Immunol. 7: 339
2. Streuli, M., 1987 J. Exp. Med. 166: 1548
3. Hall, P.A., et al. 1987. J. Clin. Pathol. 40: 151
4. Poppema, S., et al 1987. Am. J. Path. 127: 418
5. West, K.P., 1986. J. Pathol. 150: 89
6. Young, J.L., 1997. Eur. J. Immunol. 27: 2383
7. Grotjahn, C., et al., 1998, p.446. Leukocyte Antigen Workshop VI.1998. Kishimoto er al., eds. Oxford University Press
8. Poppema, S., et al., 1996. Leukemia and Lymphoma, 20, 217-222
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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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