PRODUCT INFORMATION SHEET

Monoclonal antibodies detecting human antigens

CD45

PURE	RUO	REF	IQP-124P	100 tests
FITC	IVD	REF	IQP-124F	100 tests
R-PE	IVD	REF	IQP-124R	100 tests
CyQ	IVD	REF	IQP-124C	100 tests
APC	IVD	REF	IQP-124A	100 tests
Dy-410	RUO	REF	IQP-124D	100 tests
PerCP	RUO	REF	IQP-124PC	100 tests

IVD CE ***In Vitro Diagnostic medical device***

RUO ***For Research Use Only***

Description

Clone ML2

Isotype murine IgG1

Specificity CD45 (ML2) reacts with the CD45 antigen, also known as the leukocyte common antigen (LCA) or T200 antigen, comprised of different glycoproteins ranging from 180-240 kD.

Antigen distribution

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 (RA, RB, RO, RC) on lymphocyte subpopulations, such as T cells or B cells.

Summary CD45 is a family of transmembrane protein tyrosine phosphatases critically involved in the regulation of lymphocyte activation signals. Detection of distinct isoforms can distinguish between naive T cells and memory T cells, which is of interest in patients with immunodeficiency and autoimmune diseases.

Applications CD45 (ML2) can be applied in flow cytometry for analysis of blood and bone marrow samples and in immunohistochemistry using frozen tissue sections or parrafin tissue sections. CD45 clustered antibodies have proven valuable for gate setting of lymphocytes, leukocytes, and monocytes in routine testing using flow cytometry. Combination of CD45 with CD14 antibodies in the analysis of blood (or bone marrow) samples by flow cytometry shows variable expression of these antigens on different cell populations. In peripheral blood cells, a distinction can be made between lymphocytes (CD45+++, CD14 -), monocytes (CD45++, CD14++) and granulocytes (CD45++, CD14+/-). Studies on the function of individual CD45 isoforms have shown some CD45 antibodies with potent immunosuppressive activity, suggesting that CD45 may be a useful target for drug design.

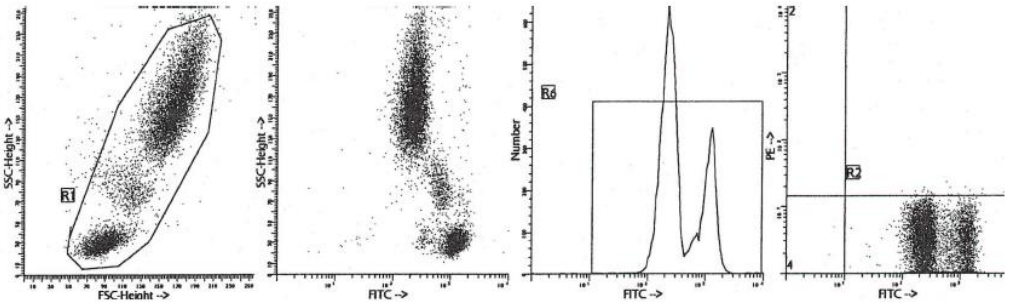
Usage All these reagents are effectively formulated for direct immunofluorescent staining of human tissue for flow cytometric analysis using 10 µl/10^6 leukocytes for singles and 20 µl/10^6 leukocytes in case of dual and triple combinations. Since applications vary, each investigator should titrate the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

HLDA Workshop

6th Leukocyte Typing Workshop - Kishimoto T., et al., Eds. Garland Pub. Inc. (1989)

Representative Data

Staining with clone ML2 (CD45) monoclonal antibodies is illustrated by flow cytometry analysis of normal blood cells. Indirect staining was performed using 10 µl of the purified monoclonal antibody with RaM FITC conjugate and 100 µl blood sample.



Reproducibility

Monoclonal antibodies from IQ Products were tested by flow cytometry using a 'lyse-wash' method on whole blood from healthy donors. Obtained data support the premise that these reagents are equivalent in their reactivity with peripheral blood lymphocytes.
Values are expressed in terms of % of the total leukocyte count (see table).

Reagent	n	Mean % positive	S.D.	% CV	Product code
CD45 FITC	10	99.43	0.66	0.66	IQP-124F
CD45 R-PE	10	99.75	0.20	0.20	IQP-124R
CD45 CyQ	10	99.33	0.35	0.35	IQP-124C
CD45 APC	10	99.72	0.30	0.31	IQP-124A

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, CyQ, APC or PerCP) 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 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) 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. Pingel, J.T. and Thomas, M.L., 1998. Cell. 58. 1055 - 1065
4. Neel, B.G., 1997. Curr. Opin. Immunol. 9: 405
5. Kishimoto et al, eds., Leukocyte Typing VI 1998. Garland Publishing Inc.

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)

	Conjugates		**Ex -max (nm)**	**Em -max (nm)**
P	PURE	Unconjugated antibody	-	-
F	FITC	Fluorescein Isothiocyanate	488	519
R	R-PE	R-Phycoerythrin	488, 532	578
C	CyQ	Tandem conjugate of R-PE-and Cy5.18	488, 532	667
A	APC	Allophycocyanin	595, 633, 635, 647	660
D	Dy-410	Violet Dye 410	405	460
PC	PerCP	Peridinin-chlorophyll-protein	488, 532	678

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