

## 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
PerCP	RUO	REF	IQP-124PC	▽	100 tests
PerCP-Cy5.5	RUO	REF	IQP-124PCC	▽	100 tests

IVD  **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 paraffin 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

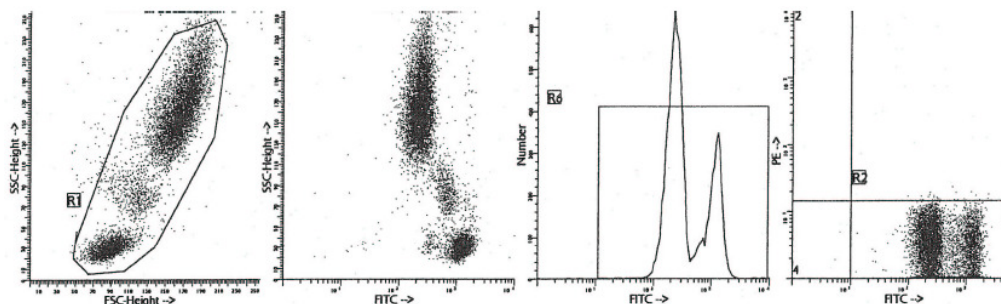
All these reagents are effectively formulated for direct immunofluorescent staining of human tissue for flow cytometric analysis using 10 µl/10<sup>6</sup> leukocytes for singles and 20 µl/10<sup>6</sup> leukocytes in case of dual and triple combinations. Since applications vary, each investigator should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

### HLDA Workshop

6<sup>th</sup> 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 Starfigs - 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<sup>6</sup> 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)<sub>2</sub> 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 ( $\text{NaN}_3$ ). 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



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)

		<b>Label - tandem</b>	<b>Ex -max (nm)</b>	<b>Em -max (nm)</b>
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	APC	595, 633, 635, 647	660
OC	OC515	OC515	405, 407	515
PC	PerCP	PerCP	488, 532	678
PCC	PerCP-Cy5.5	PerCP-Cy5.5	488, 532	695



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bright fluorescence

