PRODUCT INFORMATION SHEET

Monoclonal antibodies detecting human antigens

CD14

PURE	RUO	REF	IQP-143P	100 tests
FITC	IVD	REF	IQP-143F	100 tests
R-PE	IVD	REF	IQP-143R	100 tests
APC	IVD	REF	IQP-143A	100 tests

IVD CE ***In Vitro Diagnostic medical device***
RUO ***For Research Use Only***

Description

Clone UCHM1

Isotype Murine IgG2a

Specificity CD14 (UCHM1) recognizes the human CD14 glycoprotein (55 kD) .

Antigen distribution
CD14 is expressed on the majority of peripheral blood monocytes (approx. 80%), macrophages and weakly on granulocutes.

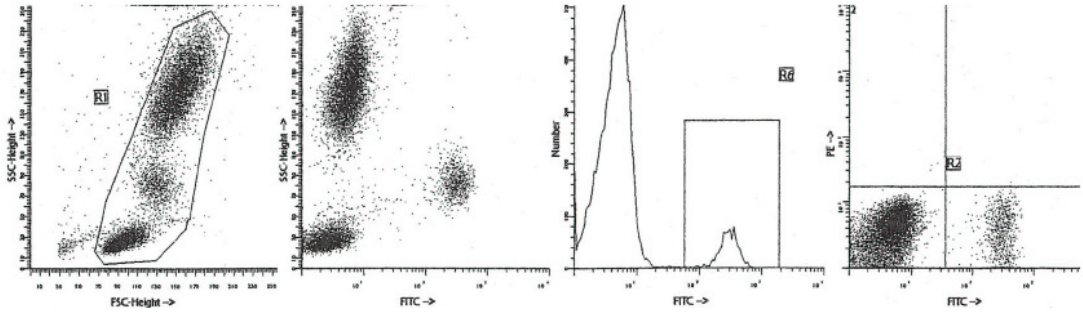
Summary In lymphoid tissue, UCHM1 reacts with monocyte-like cells, such as in the red pulp of the spleen and in the subcapsular region of thymus. Strong reactivity is found on vascular endothelium in lymph nodes and liver, weak reactivity in thymus, spleen and tonsil, and no reactivity in lung, brain, skin and kidney. CD14 is a receptor for the LPS binding protein, and may be involved in the clearance of pathogens from the blood. CD14 (UCHM1) is reactive with cell lines U937 (histiocytic lymphoma), THP1-0 (probably monocytic) and 15% of HL60 (weakly, a promyelocytic cell line), but not with K562 (erythroleukemia cell line) or other B or T cell lines.

Applications CD14 (UCHM1), can be applied in flow cytometry and in immunohistochemistry using frozen tissue sections. CD14 is suitable for the detection of peripheral blood monocytes. CD14 is often used in combination with CD45 (pan-leukocytes) antibodies for differentiation of lymphocytes, granulocytes and monocytes in analysis of peripheral blood samples using flow cytometry. It is also used for immunophenotyping of cells by flow cytometry using three color staining of broncho-alveolar lavage (BAL) samples with CD15/CD14/CD45. Neutrophilic granulocytes stain CD15++, CD45+ but show weak expression of CD14. CD14 (UCHM1), is important for analysis of acute monocytic leukemias and most leukemias are UCHM1 positive. An example is true histiocytic lymphoma (THL), which stains strongly for CD14 and a large number of monocytic markers, depending on the stage of monocyte maturation e.g. CD11b, CD11c, CD13, CD15, CD33, CD36, CDw65, HLA-DR and cytoplasmic MPO.

Usage CD14 is expressed on the majority of peripheral blood monocytes (approx. 80%), macrophages and weakly on granulocutes.

HLDA Workshop
6th Leukocyte Typing Workshop - Knapp, W., et al., Oxford University Press, New York (1989)

Representative Data
Staining with clone UCHM1 (CD14) monoclonal antibodies is illustrated by flow cytometry analysis of normal blood cells. Direct staining was performed using 10 µl of the FITC-conjugated antibody 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
CD14 FITC	9	5.61	1.32	23.64	IQP-143F
CD14 R-PE	9	5.63	1.36	24.12	IQP-143R
CD14 APC	9	6.11	1.48	24.22	IQP-143A

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 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 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

IQ Products bright fluorescence

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

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3. Hogg, N., MacDonald, S., Slusarenko, M. and Beverley, P.C.L., 1984. Immunology. 53: 753-768
4. Herrmann, F., Komischke, B., Odenwald, E., and Ludwig, W.D., 1983. Acute Myeloid Leukemia and Acute Phase of Chronic Myeloid Leukemia. Blut. 47: 157
5. Linch, D.C., Allen, C., Beverley, P.C.L., Bynoe, A.G., Scott, C.S. and Hogg, N., 1984. Blood. 63: 566-573
6. Hogg, N. and Horton, M.A., 1987. In: Leucocyte Typing III 576-602, McMichael, A.J., et al., eds Oxford University Press, NY
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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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