PRODUCT INFORMATION SHEET

Monoclonal antibodies detecting human antigens

CD4

PURE	RUO	REF	IQP-535P	100 tests
FITC	IVD	REF	IQP-535F	100 tests
R-PE	IVD	REF	IQP-535R	100 tests
CyQ	IVD	REF	IQP-535C	100 tests
APC	IVD	REF	IQP-535A	100 tests
Dy-410	RUO	REF	IQP-535D	100 tests
PerCP	RUO	REF	IQP-535PC	100 tests

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

Description

Clone Edu-2

Isotype murine IgG2a

Specificity CD4 (Edu-2) recognizes the CD4 antigen (a 55 kD glycoprotein).

Antigen distribution
The CD4 antigen is present on most thymocytes and a subpopulation of peripheral blood T cells, called T helper cells (Th). In addition, CD4 is expressed on monocytes and weak on macrophages.

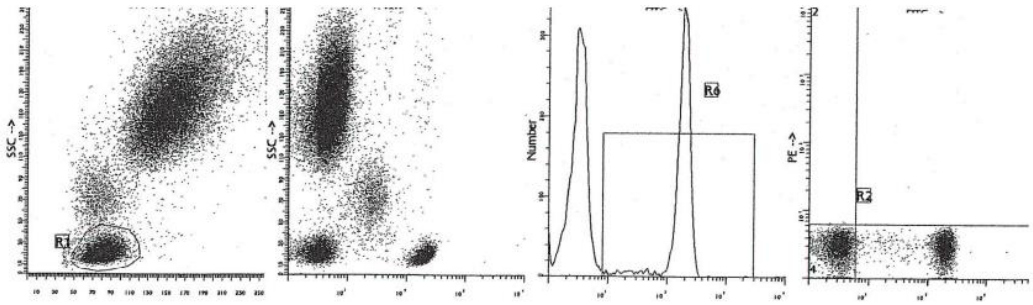
Summary CD4 plays a role in the recognition of foreign antigens presented to T cells by MHC class II molecules. Furthermore, this antigen acts as a receptor for HIV-1 by binding the viral protein gp120.

Applications Flow cytometry and immunohistochemistry using frozen and paraffin embedded tissue sections. CD4 (Edu-2) is used in routine blood testing for CD4+ cells and CD4/CD8 ratios (e.g. HIV/AIDS patients) or as part of panels for the detection and differentiation of certain T cell leukemias.
CD4 is also used in studies of functional activity of Th cells in bacterial and viral infections, development of auto-immune diseases, transplant rejection, immune protection in response to allergens or allergenic reactivity.

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
5th Leukocyte Typing - Schlossman, S.F., et al., eds. Oxford University Press, New York (1995)

Representative Data
Staining with clone Edu-2 (CD4) 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 lymphocyte count (see table).

Reagent	n	Mean % positive	S.D.	% CV	Product code
CD4 FITC	10	45.36	5.20	11.46	IQP-535F
CD4 R-PE	10	47.18	5.20	11.02	IQP-535R
CD4 CyQ	10	47.51	5.01	10.55	IQP-535C
CD4 APC	10	46.10	5.08	11.03	IQP-535A

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, PerCP or PerCP-Cy5.5) 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. Carrière, D., et al., 1995, In: Leukocyte Typing V: 475-476. S.F. Schlossman, L. Boumsell, W., et al., eds. Oxford University Press, New York
2. Piatier-Tonneau, D., et al.,1995, In: Leukocyte Typing V: 476-478. S.F. Schlossman, L. Boumsell, W. Gilks, et al., eds. Oxford University Press, New York
3. Kaneoka, H. et al., 1983. J. Immunol., 131: 158
4. Lanier, L.L., et al., 1986. J. Immunol., 137: 2501
5. Knowles, R.W., 1986. In: Leukocyte Typing II : 259-288; E.L. Reinhert, B.F. Haynes, L.M. Nadler/ and I.D. Bernstein, eds. Springer-Verlag, New York
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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)

	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

IQ Products BV
Rozenburglaan 13a
9727 DL Groningen, The Netherlands

+31 (0)50 57 57 000
+31 (0)50 57 57 002
Technical marketing@iqproducts.nl
Orders orders@iqproducts.nl
www.iqproducts.nl