PRODUCT INFORMATION SHEET

Monoclonal antibodies detecting human antigens

CD3

PURE	RUO	REF	IQP-519P	100 tests
FITC	IVD	REF	IQP-519F	100 tests
R-PE	IVD	REF	IQP-519R	100 tests
CyQ	IVD	REF	IQP-519C	100 tests
APC	IVD	REF	IQP-519A	100 tests
Dy-410	RUO	REF	IQP-519D	100 tests
PerCP	RUO	REF	IQP-519PC	100 tests

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

Description

Clone UCHT1

Isotype murine IgG1

Specificity CD3 recognizes the 20-28 kDa ε-chain of the CD3 molecule complex

Antigen distribution
The CD3 antigen is present on 70-80% of normal human peripheral blood lymphocytes and 10-20% of thymocytes. CD3 is expressed on greater than 95% of circulating human peripheral T cells.

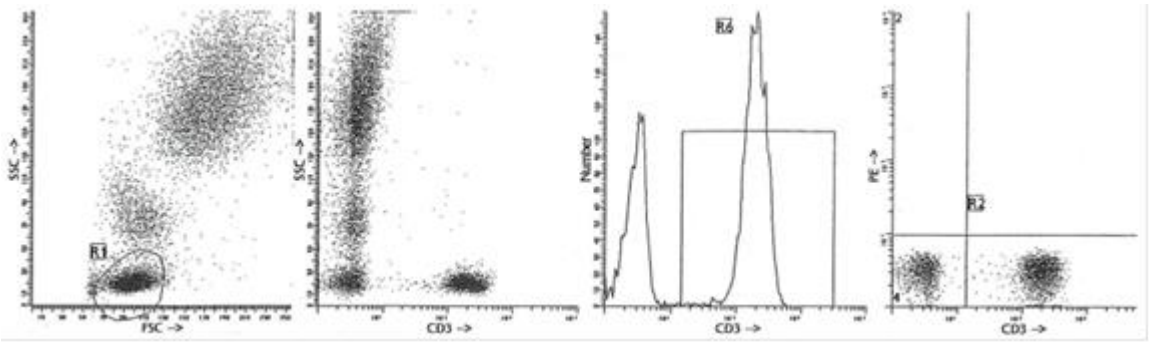
Summary CD3 has a mitogenic effect (on resting) T lymphocytes. It blocks the cytolytic activity of CTL clones. CD3 reacts with the constant structure of the CD3/T cell receptor complex (TcR). It does not react with those T cells lacking the TcR. The TcR is present on mature T cells and during thymopoiesis. This complex is made up of at least five CD3 proteins, CD3γ, CD3δ, CD3ε, the ζ-chain, and the η-chain in association with either TcRα/β or TcRγ/δ proteins. TcR recognizes antigens in association with MHC molecules after which protein chains of the CD3 complex mediate activation signals triggered by TcR antigen binding.

Applications Flow Cytometry; Activation; Blocking; ELISA; IHC on frozen sections; Immunoprecipitation.
This antibody can be used to detect intracytoplasmic CD3 (cyCD3) in flow cytometry after permeabilization.

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 - Bernard, A;, et al. Eds., Springer-Verlag (1981)

Representative Data
Staining with clone UCHT1 (CD3) 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 lymphocyte count (see table).

Reagent	n	Mean % positive	S.D.	% CV	Product code
CD3 FITC	10	66.50	3.77	5.66	IQP-519F
CD3 R-PE	10	69.18	4.11	5.94	IQP-519R
CD3 CyQ	10	71.15	2.81	3.95	IQP-519C
CD3 APC	10	72.19	4.45	6.16	IQP-519A

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, Dy-410, 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. Characterization of an antigen that is recognized on a melanoma showing partial HLA loss by CTL expressing an NK inhibitory receptor. Ikeda, H., Lethé, B., Lehmann, F., Van Baren, N., Baurain, J.F., De Smet, C., Chambost, H., Vitale, M., Moretta, A., Boon, T., Coulie, P.G. 1997.
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3. Analysis of the activation signals induced by CD3 antibodies and their role in T-cell proliferation. Wallace, DL., Mancintyre, EA., Linch, DC., Beverley, PCL. 1987.
4. Activation of human thymocytes via CD3 and CD2 molecules. Denning, S.M., Tuck, D.T., Singer, K.H., Haynes, B.F. 1987.
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6. T cells from patients with Hodgkin's disease have a defective T-cell receptor zeta chain expression that is reversible by T-cell stimulation with CD3 and CD28. Renner, C., Ohnesorge, S., Held, G., Bauer, S., Jung, W., Pfitzenmeier, Pfreundschuh, M. 1996.
7. Expression of the IL-2 receptor gamma subunit in resting human CD4 T lymphocytes : mRNA is constitutively transcribed and the protein stored as an intracellular component. Bani, L., David, D., Moreau, J.L., Cayota, A., Nakarai, T., Ritz, J., Thèze, J. 1997.
8. Positive effects of combined antiretroviral therapy on CD4+ T cell homeostasis and function in advanced HIV disease. Autran, B., Carcelain, G., Li, T.S., Blanc, C., Mathez, D., Tubiana, R., Katlama, C., Debré, P., Leibowitch, J. 1997.
9. CD designations and commercially available antibodies. Leong, A.S.Y. 1993
10. Evidences for protein kinase C. Activation in T lymphocytes by stimulation of either the CD2 or CD3 antigens. Friedrich, B., Cantrell, D.A., Gullberg, M. 1989.

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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