www.iqproducts.nl

PRODUCT INFORMATION SHEET

Monoclonal antibodies detecting human antigens

CD10 FITC **CD19** R-PE IVD REF IQP-264FR 50 tests

IVD CE *In Vitro Diagnostic medical device*

Description

CD10 *Clone* **B-E3** *Isotype* **murine IgG2a**

For detailed description of this particular single reagent, please refer to IQP-105, CD10 (B-E3)

CD19 *Clone* **HD37** *Isotype* **murine IgG1**

For detailed description of this particular single reagent, please refer to IQP-515, CD19 (HD37)

Intended use

CD10/CD19 dual combination, IQP-264FR, is a direct immunofluorescence reagent used for the characterization of leukemias and lymphomas in human lysed whole peripheral blood or mononuclear cells separated by density gradient using flow cytometry.

Summary

On CD19 positive lymphocytes from patients with acute B-lymphoid leukemia the CD10 antigen is found. Publication state that pre-ALL patients expressing CD10 have a better prognosis than CD10 negative forms of ALL. Using flow cytometry expression of the antigens can be investigated.

Applications

Human CD10 (Mw = 100kDa) or "Common Acute Lymphoblastic Leukaemia Antigen" (CALLA) is expressed on early B and T lymphoid precursors, B blasts, some granulocytes and bone marrow stromal cells. CD10 is also expressed on various epithelia, some smooth muscle and myoepithelial cells, brain and fibroblasts. CD10 is a zinc-binding metalloprotease, which is thought to down regulate cellular responses to peptide hormones by degrading the peptide bonds and reducing the concentration of peptide available for receptor binding and signal transduction.

Monoclonal antibodies clustered as CD19 (MW = 95kDa) detect all peripheral blood B cells. In addition, CD19 is expressed on precursor B cells during maturation, but not on mature plasma cells. CD19 may also be expressed on follicular dendritic cells. It is not expressed on T lymphocytes, granulocytes, activated T cells or monocytes. The function of the CD19 molecule is related to signal transfer and is involved in regulation of B cell proliferation. CD19 is considered to be a characteristic B cell marker and therefore commonly used in routine immunophenotyping. Moreover, CD19 is present on acute and chronic B cell leukemias and lymphomas.

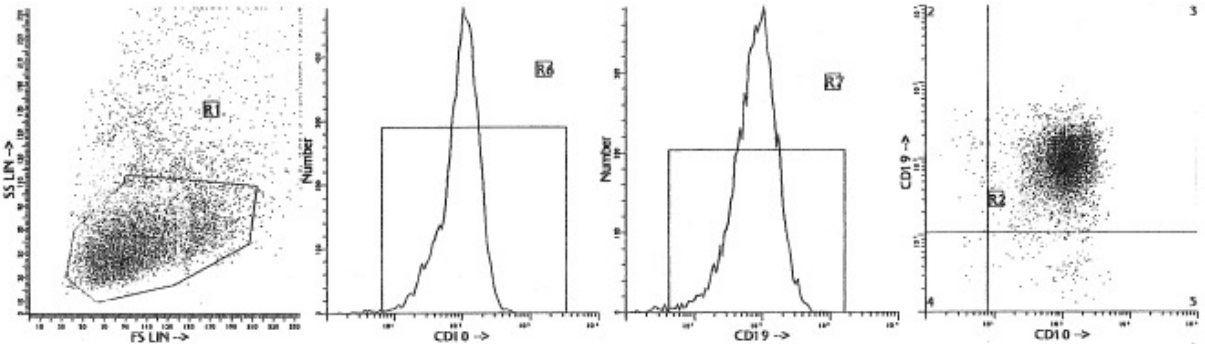
Note: Not all the applications mentioned are performed using IQ Products reagents

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.

Representative Data

Staining with clone B-E3 (CD10)/HD37 (CD19) monoclonal antibodies is illustrated by flow cytometry analysis of Nalm6 cells. Direct staining was performed using 20 µl of the dual and 100 µl cell suspension.



Reproducibility

Monoclonal antibodies from IQ Products were tested by flow cytometry using IQ Lyse (IQP-199). The used 'lyse-wash' method is 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
CD10 FITC	10	99,75	0,03	0,03
CD19 R-PE	10	13,10	4,04	30,82

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% Heparin
10. 1% paraformaldehyde solution in PBS (store at 2-8 °C in amber glass for up to 1 week)

Immunofluorescence staining and lysing protocol

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) for singles 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. Prognostic significance of the CD10+CD19+CD34+ B-progenitor immunophenotype in children with acute lymphoblastic leukemia: a report from the Children's Cancer Group. Leuk Lymphoma. 1997 Nov; 27 (5-6): 445-57
2. Poppema, S and Visser, L, In: Monoclonal Antibodies in the Characterization of Lymphomas and the Diagnosis of Disease; Proc. of the 9th Biotest Symposium, Institute of Education, London, 1987. Sonneborn HH and Tills D eds
3. Moldenhauer, G et al. In Leucocyte Typing II Human B Lymphocytes, 1986, 61-67. EL Reinherz, HF Haynes, LM Nadler, and ID Bernstein eds (Springer-Verlag, New York)
4. Meeker, TC et al, 1984. Hybridoma. 3:305
5. Loken, MR et al, 1987. Blood 70:1316
6. Rothe, G, and Schmitz, G. Leukemia, 1996. 10:877-895

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

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