

PRODUCT INFORMATION SHEET

Monoclonal antibodies detecting human antigens

CD20

PURE	RUO	REF	IQP-108P	▽	100 tests
FITC	IVD	REF	IQP-108F	▽	100 tests
R-PE	IVD	REF	IQP-108R	▽	100 tests
APC	IVD	REF	IQP-108A	▽	100 tests
Dy-410	RUO	REF	IQP-108D	▽	100 tests
PerCP	RUO	REF	IQP-108PC	▽	100 tests

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



Description

Clone B-ly1

Isotype murine IgG1

Specificity CD20 (B-ly1) recognizes a 33-37 kD human B cell antigen.

Antigen distribution

CD20 is expressed only on B lineage cells but is absent from plasma cells. It is found on approximately 10% of human peripheral blood lymphocytes and in high density in B cell areas of lymphoid tissue.

Summary

CD20 is expressed by pre-B cells in the bone marrow, presumably after Ig heavy chain rearrangement, and expression persists during all stages of B-cell maturation but is lost on terminal differentiation to plasma cells. It is expressed by the majority of B-cell lineage malignancies, including half of acute lymphoblastic leukemias. No extracellular ligand for CD20 has been identified. There are reports that CD20 regulates cell cycle progression and exists on the cell surface as a homo-oligomer. Evidence suggests that CD20 functions as a B cell Ca²⁺-channel subunit, since the expression of CD20 in various cell types generates a similar type of channel activity to that found endogenously in B cells. It is thought that CD20 is a central component of a multimeric molecular complex that plays a regulatory role in B-lymphocyte activation, proliferation and differentiation.

Usage

All these reagents are effectively formulated for direct immunofluorescent staining of human tissue for flow cytometric analysis using 10 µl/10⁶ leukocytes for singles and 20 µl/10⁶ leukocytes in case of dual and triple combinations. Since applications vary, each investigator should titrate the reagent to obtain optimal results.

Applications

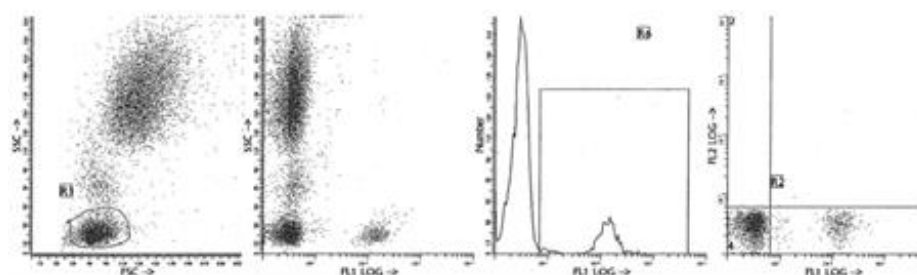
CD20 (B-ly1) can be applied in flow cytometry for analysis of blood and bone marrow samples or in immunohistochemistry on frozen or paraffin embedded tissue sections. CD20 (B-ly1), is suitable for use in immunohistochemistry for the staining of B cells in tissue sections and the diagnosis of B cell lymphomas. B-ly1 is used in flow cytometry for the enumeration of B cells in peripheral blood and the analysis of B cell lymphomas and leukemias.

HLDA Workshop

4th Leukocyte Typing Workshop - Knapp, W., et al., Oxford University Press, New York (1990).

Representative Data

Staining with clone B-ly1 (CD20) 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
CD20 FITC	9	10.25	2.25	21.93	IQP-108F
CD20 R-PE	9	9.81	2.37	24.12	IQP-108R
CD20 APC	9	10.98	2.26	20.60	IQP-108A

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 Starfix - 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



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 (NaN₃). 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. Tedder, T.F., and Engel, P., 1994. Immunol today 15. 450-454
 2. Barclay, A., et al. 1997. Leukocyte antigen FactsBook. 181-182
 3. Bubien, J.K., et al. 1993. J.Cell Biol. 121. 1121-1132
 4. Kanzaki, M., et al. 1995. J. Biol. Chem. 270. 13099-13104
 5. Poppema, S., and Visser, L., 1987 Biotest Bulletin 3: 131-139
 6. Knapp, W., et al. eds. 1990. Leukocyte Typing Workshop. Oxford University Press
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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)

	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
PCC	PerCP-Cy5.5	Tandem conjugate of PerCP-and Cy5.5	488, 532	695



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