PRODUCT INFORMATION SHEET

Monoclonal antibodies detecting human antigens

CD23

PURE	RUO	REF	IQP-154P	100 tests	REF	IQP-154P50	50 tests
R-PE	RUO	REF	IQP-154R	100 tests	REF	IQP-154R50	50 tests

RUO ***For Research Use Only***

Description

Clone B-G6

Isotype Murine IgG1

Specificity Clone B-G6 produces mouse IgG1 immunoglobulins directed against CD23 antigen. It is suitable for use in flow cytometry and immunohistochemistry.

Antigen distribution

CD23 is a low affinity receptor for IgE (FceR) and both the FceRIIa and FceRIIb are expressed on mature IgD+/IgM+ B cells. The FceRIIb form is also present on monocytes, activated macrophages, eosinophils, platelets and follicular dendritic cells [1]. CD23 is also present in a soluble form in serum [2]. CD23 together with CD19 and CD22, plays a role in the modulation of B cell activation through membrane immunoglobulin and is involved in regulation of IgE synthesis. Expression of CD23 is increased by IL-4 and down-regulated by B-cell growth factor (BCGF). Binding of IgE immune complexes to CD23 increases the efficiency of B cell antigen processing and presentation. Via its interaction with CD21 it may play a role in adhesion processes. It mediates IgE-dependent cytotoxicity and phagocytosis by macrophages and eosinophils [3,4].

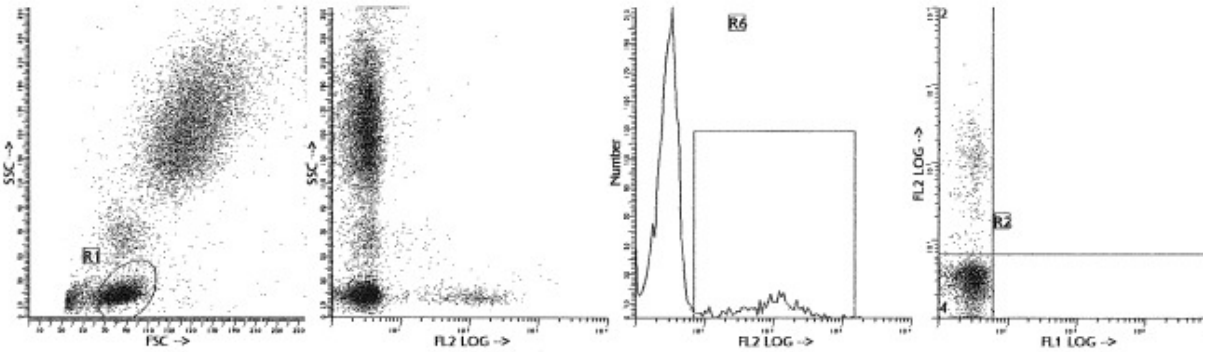
Summary CD23 is used in studies of B lymphocyte differentiation, and for measurement of Fce receptors in plasma. Most mature IgD+/IgM+ B cells which express CD23, lose this antigen after isotype switching to the downstream isotypes a, g, or e. It is not present on immature bone marrow lymphocytes or T lymphocytes. Its expression is strikingly increased during the B-cell activation process following membranous (m)Ig ligation and IL-4 stimulation or T-B interactions. CD23 binds to the surface adhesion molecules CD11b/CD11c expressed on monocytes and results in the upregulation of pro-inflammatory cytokines such as IL-6, IL-1b and TNF-a [5].
The CD23 antigen is present at low levels on most normal B lymphocytes and is more strongly expressed on activated B cells, Epstein-Barr virus (EBV) transformed lymphoblasts, and chronic lymphocytic leukemia (CLL) cells of B-lymphocyte origin. It is absent in lymphoblastic lymphoma or leukemia, centrocytic lymphoma, hairy cell leukemia and T cell lymphoma.

Applications Monoclonal antibody clone B-G6 (CD23) can be applied in flow cytometry for analysis of blood and bone marrow samples, or in immunohistochemistry using cytospots or frozen tissue sections.

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-G6 (CD23) is illustrated by flow cytometry analysis of normal blood cells. Direct staining was performed using 10 µl of R-PE conjugated antibody with 100 µl blood sample.



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, Cy-Q 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) resp. 50 tests per vial (0.5 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. Conrad, D.H., 1990 Annu. Rev. Immunol. 8.623-645
2. Richards, M.L. et al. 1991 Critic. Rev. Immunol. 11.65-86
3. Delespesse, G, et al. 1992 Immunol. rev. 125. 77
4. Flores-Romo, L, et al. 1993 Science, 2261,1038
5. Lecoanet-Henchoz, S., et al. 1997 Eur. J. Immunol. 27. 2290-2294

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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