PRODUCT INFORMATION SHEET

Monoclonal antibodies detecting human antigens

CD33

PURE	RUO	REF	IQP-572P	100 tests
R-PE	RUO	REF	IQP-572R	100 tests
APC	RUO	REF	IQP-572A	100 tests

RUO ***For Research Use Only***

Description

Clone 251

Isotype murine IgG1

Specificity 251 detects the CD33 antigen, a 67 kDa molecular weight protein.

Antigen distribution

251 reacts with the CD33 antigen expressed on human peripheral blood monocytes and weakly on granulocytes. Expression is also found on myeloid progenitor cells, such as granulocyte and macrophage precursor cells in bone marrow. No reactivity has been found with normal lymphocytes, erythrocytes and platelets, nor with pluripotent stem cells. In addition, 251 reacts with myeloid cell lines HL-60, KG-1, and U937.

Summary CD33 antigen is an important marker for detection of normal myeloid cells and their malignant counterparts. CD33 can be applied in the classification of leukemias.

Applications 251, is applied in flow cytometry for analysis of blood and bone marrow samples, or in immunohistochemistry using frozen tissue sections. Immunohistochemical application includes reactivity with histiocytes, a percentage of Langerhans cells and perivascular macrophages of tissue sections such as in skin.
Discrimination of subtypes of acute leukemias can be performed using 251. For example, CD33 is used in combination with CD11b and CD13 antibodies. Both antigens are present on most myeloid leukemias (AML, acute myeloid leukemia), myelo-monocytic leukemias and pro-myelocytic leukemias. In contrast, erythroblastic acute leukemia and megakaryo-blastic leukemia, show no presence of these two antigens. CD33 is generally not expressed on ALL, like T-ALL (Acute Lymphoblastic Leukemia) and B-ALL, and is therefore useful as a myeloid leukemia defining cell marker antigen. However, studies performed in a large population of acute leukemia patients resulted in CD33 positive cases within the precursor B (common) form of ALL. Interestingly, the CD33 antigen is not present on CD34+ stem cells, while further differentiated cells, e.g. progenitor cells of granulocytes, monocytes/macrophages, erythroid cells and cells of megakaryocytic lineage, do express CD33. Therefore, CD33 antibodies may be applied in bone marrow purging of AML for autologous bone marrow transplantation.

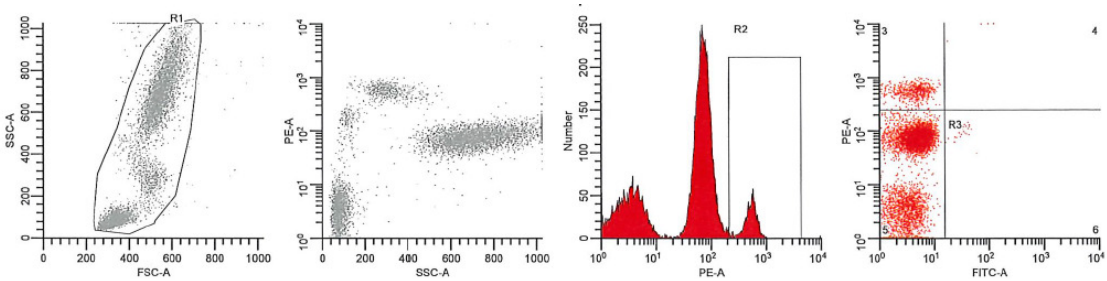
Usage All these reagents are effectively formulated for direct immunofluorescent staining of human tissue for flow cytometric analysis using 10 µL/10^6 leukocytes for singles and 20 µL/10^6 leukocytes in case of dual and triple combinations. Since applications vary, each investigator should titrate the reagent to obtain optimal results.

HLDA Workshop

6th Leukocyte Typing Workshop - Kishimoto T, et al., eds. 1998

Representative Data

Staining with clone 251 (CD33) monoclonal antibodies is illustrated by flow cytometry analysis of normal blood cells. Direct staining was performed using 10 µl of the R-PE-conjugated antibody and 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, 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) 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 Kishimoto T, Kikutani H, von dem Borne AE. G. Kr, et al. Leucocyte Typing VI: White Cell Differentiation Antigens. New York: Garland Publishing; 1998.
2 Maliszewski CR, Ball ED, Graziano RF, Fanger MW. Isolation and characterization of My23, a myeloid cell-derived antigen reactive with the monoclonal antibody AML-2-23. J Immunol 1985;135(3):1929-1936.
3 Silla LM, Chen J, Zhong RK, Whiteside TL, Ball ED. Potentiation of leukaemia cells by a bispecific antibody to CD33 and CD16 (Fc gamma RIII) expressed by human natural killer (NK) cells. Brit J Haematol 1995;89(4):712-718.
4 Balaian L, Ball ED. Inhibition of acute myeloid leukemia cell growth by monospecific and bispecificanti-CD33 x anti-CD64 antibodies. Leukemia Res 2004;28(8): 821-829.

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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