PRODUCT INFORMATION SHEET

Monoclonal antibodies detecting human antigens

CD34

PURE	RUO	REF	IQP-144P	100 tests	REF	IQP-144P50	50 tests
FITC	IVD	REF	IQP-144F	100 tests	REF	IQP-144F50	50 tests
R-PE	IVD	REF	IQP-144R	100 tests	REF	IQP-144R50	50 tests
APC	IVD	REF	IQP-144A	100 tests	REF	IQP-144A50	50 tests

RUO ***For Research Use Only***

IVD CE ***In Vitro Diagnostic medical device***

Description

Clone 581

Isotype murine IgG1

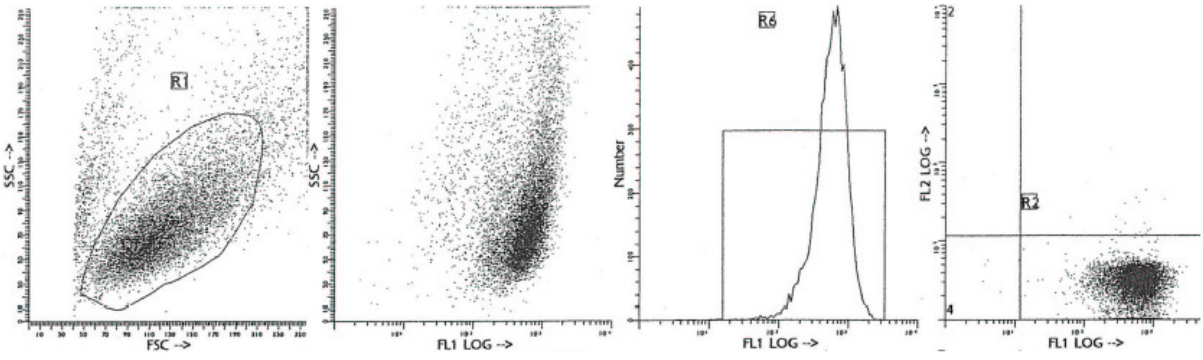
Specificity CD34 (581) reacts with the human CD34 antigen. The CD34 antigen is a heavily glycosylated membrane protein of unknown function with no homology to any other known proteins.

Antigen distribution
CD34 is expressed on haematopoietic progenitor cells, vascular endothelium, and some tissue fibroblasts.

Summary CD34 antibodies are important in experimental and clinical studies on human haemopoietic progenitors, candidate stem cells, and leukemic cell populations. CD34 antibodies are used clinically for bone marrow manipulation. It appears that the glycosylation of CD34 differs between HEV, vascular endothelium or haemopoietic cell lines, suggesting the existence of HEV-specific glycoforms of CD34 that could function as HEV ligands of lymphocyte L-selectin in humans. This suggests that, in addition to its role in haemopoiesis, CD34 could also play a role in lymphocyte recirculation.

Applications CD34 (581), can be applied in flow cytometry for analysis of blood and bone marrow samples or in immunohistochemistry using cytospots or frozen tissue sections. Not suitable for paraffin sections. CD34 antibodies can be divided into 3 major groups depending on the CD34 epitope they react with. CD34 (581) recognizes the same epitope as clone HPCA-2 and shows identical staining in flow cytometry analysis. 581 reacts with the CD34+ cell line KG1a, and CD34 transfected COS cells, CD34+ murine L cells and CD34 protein coated beads but does not recognize CD34 glycoprotein in Western blots. This antibody belongs to the group of CD34 antibodies which recognizes an epitope that is sensitive to denaturation. However, this same epitope is resistant to neuraminidase, chymotrypain and glycoprotease as shown by the good staining of CD34 positive cells with CD34 Mab after treatment with these enzymes. Immunohistochemical staining with 581 detects the luminal surface of the endothelium of capillaries and large vessels, as well as fibroblasts in the upper dermis or associated smooth muscle cells. Reactivity is lost in paraffin sections. 581 is among the group of CD34 antibodies which reacts most strongly with the HEV of lymphoid organs. The variation in epitope expression reflects to some degree the maturation stage of the leukemia, and may be important for the sub-classification of CD34+ acute leukemias. 581 was found to react strongly with a panel of blast cells from four AML (FAB classification 1 to 4) and two ALL samples (an early B-lineage and an early T-lineage leukemia).

Representative Data
Staining with clone 581 (CD34) monoclonal antibodies is illustrated by flow cytometry analysis using KG1a cells. Direct staining was performed using 10 µl of the FITC-conjugated antibody and 100 µl KG1a cells.



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

5^{th} Leukocyte Typing Workshop - Gaudernack, G., Egeland, T. (1995)

Reproducibility

Monoclonal antibodies from IQ Products were tested by flow cytometry using a 'lyse-wash' method on KG1a cells. Obtained data support the premise that these reagents are equivalent in their reactivity with these cells. Values are expressed in terms of % of the total cell count (see table).

Reagent	Mean % positive	S.D.	% CV	Product code
CD34 FITC	99,36	0,21	0,22	IQP-144F
CD34 R-PE	99,60	0,16	0,16	IQP-144R
CD34 APC	100,00	0,00	0,00	IQP-144A

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) 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. Civin, C., et al., 1984, J. Immunol.133: 157
2. Tindle, R.W., et al., 1984. Leuk. Res. 9: 1
3. Andrews, R.G., et al., 1986. Blood 67: 842
4. Fina, L., et al. 1990. Blood. 75: 2417
5. Schilingemann, R.O., et al., 1990. Lab.Invest. 62: 690
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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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