PRODUCT INFORMATION SHEET

Monoclonal antibodies detecting human antigens

CD235a

PURE	RUO	REF	IQP-145P	100 tests
FITC	RUO	REF	IQP-145F	100 tests

RUO ***For Research Use Only***

Description

Clone NaM10-6G4

Isotype Murine IgG2a

Specificity Clone NAM10-6G4 produces mouse IgG2a immunoglobulins directed against the human CD235a antigen (41 kD) which is expressed as a cell surface sialoglycoprotein.

Antigen distribution

Glycophorin A (GpA) antigen (CD235a) is detected by the monoclonal antibody NAM10-6G4 and is exclusively expressed on human erythroid cells and their progenitors. These antibodies may be effectively used for differentiation in acute leukemias e.g. presence of the GpA antigen on leukemia cells indicates an early erythroid cell lineage of the tumor cells.

Summary CD235a is not expressed on lymphoid or granulocytic progenitor cells and therefore is very useful as a marker for detection of the erythroid cell lineage [1,2]. CD235a seems to be absent from erythroid colony forming cells in bone marrow, but is present during maturation from (pro-)erythroid blast cells to mature erythrocytes. CD235a is clinically important in the classification of leukemias. Using CD235a antibodies, a distinction can be made between erythroleukemia and other (acute) leukemias like e.g. myeloid, lymphoid or undifferentiated leukemias [3,4]. CD235a is often used in combination with detection of H-antigen and/or anti-CD36 antibodies for additional characterization of early erythroid cells. Additional immunophenotyping of early erythroleukemias can be performed by detecting myeloid-associated antigens such as CD13, CD14, CD15, CD33 and CD34, to discriminate from lymphoid lineage-associated antigens like CD2, CD7, CD10 and CD19 [6].

Applications CD235a antibodies, clone NAM10-6G4, can be applied in flow cytometry for analysis of blood and bone marrow samples or in immunohistochemistry using frozen tissue sections. CD235a antibodies are used to identify erythroid cells during hematopoietic differentiation.

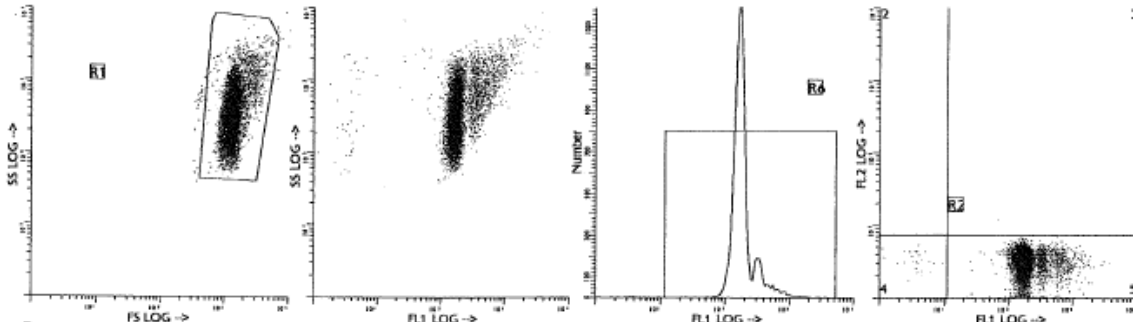
Usage All these reagents are effectively formulated for direct immunofluorescent staining of human tissue for flow cytometric analysis using a diluted blood sample (100x) from a healthy human volunteer. 10 µl of the FITC-conjugated monoclonal antibody preparation is used for 100 µl of the diluted blood sample. Since applications vary, each investigator should titrate the reagent to obtain optimal results.

HLDA Workshop

7th International Workshop on Human Leukocyte Differentiation Antigens, Harrogate.

Representative Data

NAM10-6G4 (CD235a) was analyzed by flow cytometry using a diluted blood sample (100x) from a healthy human volunteer. Direct staining was performed using 10 µl of the FITC-conjugated monoclonal antibody preparation and 100 µl of the diluted blood sample.
N.B. No lysing solution was used.



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. PBS (phosphate-buffered saline)
7. PBS (phosphate-buffered saline) containing heparin
8. Dextran solution

Erythrocytes

Preparation of Red Blood Cell Suspension and Immunofluorescent Staining

- Mix 2.5 ml blood with 0.75 ml Dextran solution and incubate 20 minutes at 37 °C (45° angle).
- Transfer the leukocyte-containing supernatant to another tube.
- Wash the erythrocytes with PBS (containing heparin).
- Centrifuge for 10 minutes at 2000 rpm.
- Repeat this step two times.
- Prepare an erythrocyte suspension 10% in PBS containing heparin.
- Incubate 10 µl erythrocyte suspension with 100 µl monoclonal antibody for 30 minutes at room temperature in the dark.
- Add 2 ml PBS containing heparin and centrifuge 2 min at 2000 rpm.
- Repeat this step.*
- Remove the supernatant and resuspend the cells in 200 µl PBS.

*

- In case of purified monoclonal antibodies:
- 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 heparin to the tube. It is recommended that the tube is protected from light.
- Incubate for 15 minutes at room temperature in the dark.
- Add 2 ml PBS containing heparin and centrifuge 2 min at 2000 rpm.
- Remove the supernatant and resuspend the cells in 200 µl PBS.

IQ Products® bright fluorescence

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. Andersson, L.C. et al. 1979. Glycophorin A as a cell surface marker of early erythroid differentiation in acute leukemia. Int.J.Cancer, 23, 717-720
2. Loken, M.R., et al., 1987. Flow cytometric analysis of human bone marrow: I. Normal erythroid development. Blood, 69, 255-263
3. Liszka, K. et al., 1983, Glycophorin A expression in malignant hematopoiesis. Am.J.Hematol., 15, 219-226
4. Villeval, J.L. et al., 1986, Phenotype of early erythroblastic leukemias. Blood, 68, 1167-1174
5. Cuneo, A., et al. 1990. Morphologic, immunologic and cytogentic studies in erythroleukemia: evidence for multilineage involvement and identification of two distinct cytogenetic-clinicopathological types. Br.J.Hematol., 75, 346-35
6. D. Blanchard, et al. 2001. Characterization of monoclonal antibodies directed to human red blood cell glycophorins A and B. in *"Leucocyte Typing VII. White Cell Differentiation Antigens*".
8. Reid ME, et al. 2002. Epitope determination of monoclonal antibodies to glycophorin A and glycophorin B. Coordinator's report. Antibodies to antigens located on glycophorins and band 3. Trans. Clin. Biol. 9: 63-72.

IQ Products bright fluorescence

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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