PRODUCT INFORMATION SHEET

Monoclonal antibodies detecting human antigens

CD61

PURE	RUO	REF	IQP-188P	100 tests
FITC	RUO	REF	IQP-188F	100 tests
R-PE	RUO	REF	IQP-188R	100 tests

RUO ***For Research Use Only***

Description

Clone NaM28-7D6

Isotype Murine IgG2a

Specificity Clone NaM28-7D6 produces mouse IgG2a immunoglobulins directed against a 105 kD antigen.

Antigen distribution

CD61 antigen is expressed on platelets, megakaryocytes, monocytes, macrophages and endothelial cells. CD61 is the b3 integrin subunit which combines with CD41 to form the platelet glycoprotein IIb/IIIb (integrin aIIbb3) and with CD51 to form the vitronectin receptor (integrin αVβ3) [1].

Summary CD41/CD61 is the major integrin on platelets and is important for platelet adhesion and aggregation. The ligands for CD41/CD61 include fibrinogen, von Willebrand factor, fibronectin and thrombospondin. Binding to these ligands depends on the activation state of the platelets.

CD51/CD61 acts as an activation-independent receptor for platelet attachment and spreading on vitronectin. Other ligands include fibrinogen, fibronectin, von Willebrand factor, laminin and thrombospondin. CD51/CD61 may also mediate cell-cell adhesion via an interaction with CD31. Antagonists of CD51/CD61 have been shown to inhibit tumor growth by disrupting angiogenesis [3].

Applications CD61, clone NaM28-7D6, can be applied in flow cytometry for analysis of blood and bone marrow samples or in immunohistochemistry using cytospots or frozen tissue sections. CD61 antibodies are applied in the diagnosis of Glanzmann thrombastemia and leukemia.

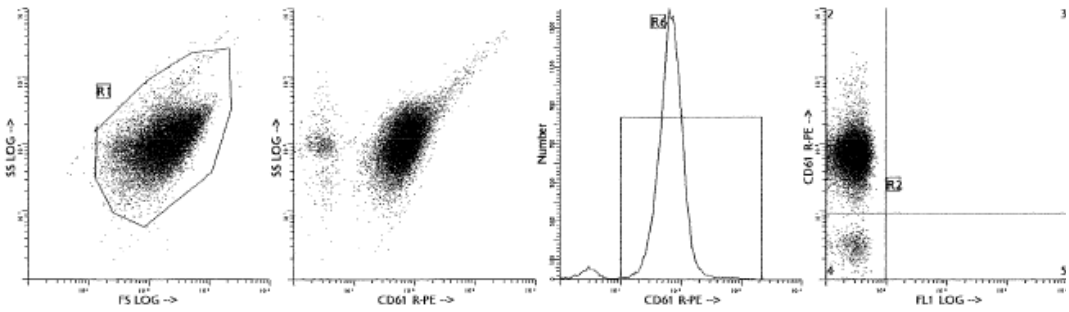
Usage All these reagents are effectively formulated for direct immunofluorescent staining of human tissue for flow cytometric analysis using 10 µl/10^6 leukocytes for singles and 20 µl/10^6 leukocytes in case of dual and triple combinations. Since applications vary, each investigator should titrate the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

HLDA Workshop

Clone NaM28-7D6 was clustered at the Leukocyte Typing Workshop VI [2].

Representative data

Clone NaM28-7D6 (CD61) was analyzed by flow cytometry using a preparation of concentrated thrombocytes. The cytogram shows direct staining with 10 µl of CD61 R-PE and 100 µl cell suspension.



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

1. Use EDTA blood.
2. Centrifuge the tube 10 minutes (600g). If possible soft start/soft stop procedure.
3. Collect plasma and dilute in PBS (+5 mM EDTA). Total volume is 10 ml.
4. Centrifuge the tube 10 minutes (2000g). If possible soft start/soft stop procedure
5. Count the cell population on a counter.
6. Dilute to $1*10^9$ cells/ml in PBS (+5 mM EDTA).
7. Add to 10 µl of cell suspension 5 µl of human serum (pooled).
8. Add 10 µl of the labeled antibody.
9. Incubate for 30 minutes at room temperature.
10. Add 2 ml of PBS (+5 mM EDTA).
11. Centrifuge the tube 5 minutes (2000g). If possible soft start/soft stop procedure.
12. Discard supernatant.
13. Resuspend the pellet in 300 µl PBS (+5 mM EDTA).
14. Choose log amplification for morphological parameters FSC/SSC.

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. Barclay A.N., et al 1997 The Leukocyte Antigen Factsbook. Academic Press. London
2. Kishimoto, et al., 1996. Leukocyte Typing VI. Kobe
3. Ginsberg, M.H., et al., 1993. Thromb.Haemost. 70. 87-93

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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