PRODUCT INFORMATION SHEET

Monoclonal antibodies detecting human antigens

CD59 FITC **CD55** R-PE RUO REF IQP-273FR 50 tests

RUO ***For Research Use Only***

Description

CD59 can be found in bodily fluids including blood plasma, saliva, amniotic fluid, seminal fluid, and urine. Since CD59 is well known membrane-associated complement regulator protein, like CD55, and present on all blood cells, CD55 and CD59 appear to be the most effective Mabs to detect very minor negative cell subsets (less than 1% on erythrocytes or less than 5% on PMN leukocytes.

CD59

Clone NaM172-2B5

Isotype Murine IgG1

Specificity CD59, Clone NaM172-2B5, can be applied in flow cytometry for analysis of blood and bone marrow samples or in immunohistochemistry using cytospots or frozen tissue.

Genetic defects in GPI-anchor attachment that cause a reduction or loss of CD59 and CD55 on erythrocytes produce the symptoms of the decease paroxysmal hemoglobinuria (PNH). CD59 does not block the lytic activity of perforin by cell-mediated cytotoxicity. It is unlikely that CD59 is synthesized by all cells on which it is expressed.

Clone NaM172-2B5, produces mouse IgG1 immunoglobulins recognizes the human CD59 antigen also known as MIRL or MACIF. CD59 is expressed as a 18-25 kD glycoprotein (in lymphocytes) anchored in the membrane by GPI tail.

CD55

Clone NaM16-4D3

Isotype Murine IgG2a

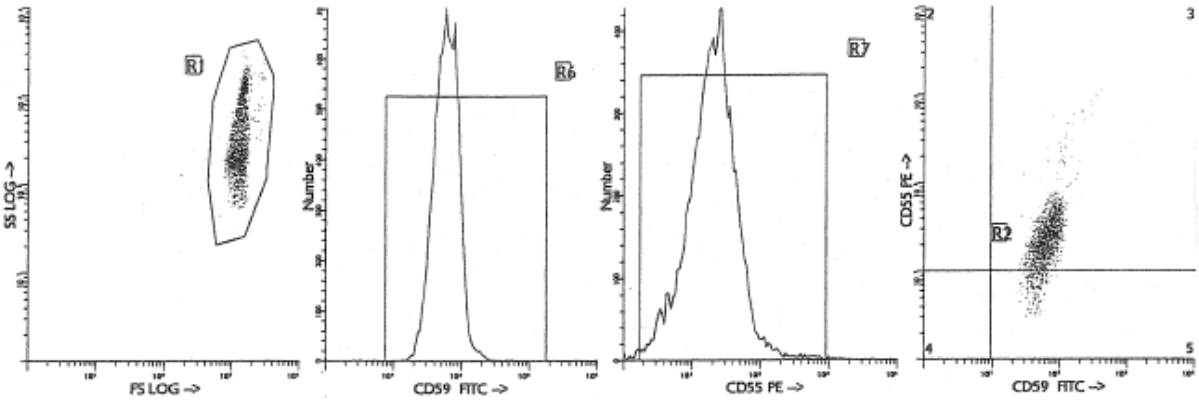
Specificity CD55, Clone NaM16-4D3, can be applied in flow cytometry for analysis of blood and bone marrow samples or in immunohistochemistry using cytospots. CD55 is a single chain Glycosyl-phosphatidylinositol (GPI) anchored glycoprotein. It is comprised of four N-terminal short consensus repeats (SCR) modules (4 Cys in 1-3,2-4 linkage) with C3b/C4b binding and regulator activity in SCR 2,3,4. CD55 is often involved in the protection of cells from autologous compliment-mediated injury and are partially or completely lacking in peripheral blood cells. CD55 inhibits the formation and accelerates the decay of C3/C5 convertase complexes both of classical and alternative pathway. CD55 sets a protective barrier threshold against inappropriate complement activation and deposition on plasma membranes, especially by the classical pathway of complement activation, by limiting formation and half-life of the C3 convertases. Since CD55 is well known membrane-associated complement regulator protein, like CD59, and present on all blood cells, CD55 and CD59 appear to be the most effective Mabs to detect very minor negative cell subsets (less than 1% on erythrocytes or less than 5% on PMN leukocytes.

Clone NaM16-4D3 produces mouse IgG1 immunoglobulins recognizes the human CD55 antigen. CD55 is expressed as a 60-70 kD glycoprotein (in erythrocytes) anchored in the membrane by GPI tail. In other cells the molecular weight is somewhat larger.

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 the dual CD59 FITC and CD55 R-PE was performed using 20 µl of the conjugated monoclonal antibody and 100 µl of red blood cell suspension.



Immunofluorescence staining and lysing protocol

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 can 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.

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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