PRODUCT INFORMATION SHEET

Monoclonal antibodies detecting human antigens

CD59

FITC	RUO	REF	IQP-521F	100 tests	REF	IQP-521F50	50 tests
R-PE	RUO	REF	IQP-521R	100 tests	REF	IQP-521R50	50 tests
APC	RUO	REF	IQP-521A	100 tests	REF	IQP-521A50	50 tests

RUO ***For research use only***

Description

Clone NaM172-2B5

Isotype Murine IgG1

Specificity 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.

Antigen distribution

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.

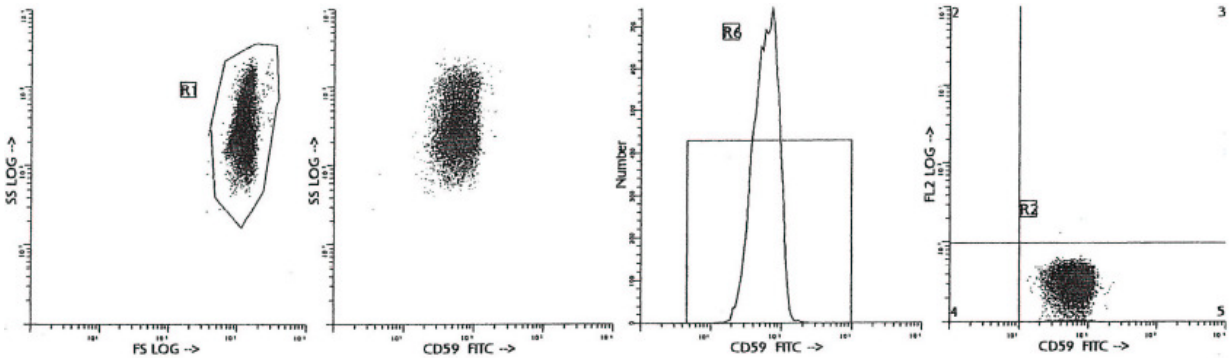
Summary Genetic defects in GPI-anchor attachment that cause a reduction or loss of CD59 and CD55 on erythrocytes produce the symptoms of the decease paroxysmal hemoglobinuria (PNH). CD59 does not block the lytic activity of perforin by cell-mediated cytotoxicity. It is unlikely that CD59 is synthesized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

Applications CD59 can be applied in flow cytometry for analysis of blood and bone marrow samples or in immunohistochemistry using cytospots or frozen tissue.

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 CD59 monoclonal antibodies is illustrated by flow cytometry analysis of normal blood cells. Direct staining was performed using 10 µl of the FITC-conjugated antibody and 100 µl red blood cell suspension.



Diagnosis of Paroxysmal nocturnal hemoglobinuria (PNH)

Procedure

Erythrocytes

-A- Preparation of Red Blood Cell Suspension

1. Use 10 ml of Heparin or EDTA whole blood and centrifuge 10 min. 600g (soft start/stop).
2. Collect the platelet rich plasma (PRP) and the buffy coat for further analysis of leukocytes and platelets, respectively.
3. Wash the pellet of erythrocytes three times with 2 ml of PBS for 2 min. 1000g.
4. Resuspend 1 volume of packed erythrocytes in 9 volumes of PBS.
5. Use a hemocytometer or automatic cell counter to calculate the total number of RBCs per ml blood collected in Heparin or EDTA treated tubes.
6. Dilute the counted RBCs with PBS to a final concentration of $50x10^6$ cells/ml.

-B- Immuno-fluorescent Staining

7. Determine the needed amount of tubes (negative control (= isotype control), positive control (= e.g. anti-glycophorin A+B), CD55 and CD59 single or dual experiments).
8. Add 100 µl of RBCs to each tube ($5x10^6$ cells).
9. Add 10 µl of the singles (CD55, CD59) or 20 µl of the dual.
10. Incubate for 30 min. at room temperature. Avoid direct light.
11. Wash twice in 3 ml PBS and centrifuge for 2 min. 1000g.
12. Resuspend the cells in PBS (200-500 µl).

-C- Flow Cytometry Data Acquisition

13. List mode files of 20.000 events should be collected for log FSC, log SSC and log fluorescence signals.

Leukocytes

-A- Preparation of Leukocyte Cell Suspension

1. Use 10 ml of Heparin or EDTA whole blood and centrifuge 10 min. 600g (soft start/stop).
2. Collect the platelet rich plasma (PRP) for further analysis of platelets.
3. Collect the buffy coat and add 10 ml of lysis buffer.
4. Incubate 5 min. at room temperature (maximum 10 min.).
5. Centrifuge 5 min. 400g to remove the lysis buffer.
6. Wash the pellet of leukocytes twice with 10 ml of PBS for 5 min. 400g.
7. Resuspend the pellet of leukocytes in 1 ml of PBS.
8. Use a hemocytometer or automatic cell counter to calculate the total number of leukocytes per ml blood collected in Heparin or EDTA treated tubes.
9. Dilute the counted leukocytes with PBS to a final concentration of $20x10^6$ cells/ml.

-B- Immuno-fluorescent Staining

10. Determine the needed amount of tubes (negative control (= isotype control), positive control (= e.g. anti-HLA class I), CD55 and CD59 single or dual experiments).
11. Add 100 µl of leukocytes to each tube ($2x10^6$ cells).
12. Add 10 µl of the singles (CD55, CD59) or 20 µl of the dual.
13. Incubate for 30 min. at room temperature. Avoid direct light.
14. Wash twice in 3 ml PBS and centrifuge for 4 min. 400g.
15. Resuspend the cells in PBS (200 – 500 µl).

-C- Flow Cytometry Data Acquisition

16. Analyze at least 20.000 cells with the flow cytometer. Use gates based on morphological parameters in order to eliminate cell debris and electronic background and to separate lymphocytes, monocytes and granulocytes.

Platelets

Prepare PBS-EDTA 5 mM pH 7.4 (50 - 75 ml per patient). For best results 0,45 µm filtered PBS-EDTA 5mM should be used. The PBS-EDTA 5 mM should be fresh (to be used during the running week) and must be filtrated before each experiment.

-A- Preparation of Platelet Cell Suspension

1. Use 10 ml of Heparin or EDTA whole blood and centrifuge 10 min. 600g (soft start/stop).
2. Collect the platelet rich plasma (PRP) and dilute in PBS-EDTA 5 mM, to a volume of 10 ml.
3. Centrifuge, 5 min. 2000g.
4. Discard supernatant and resuspend the pellet in 1 ml PBS-EDTA 5 mM.
5. Use a hemocytometer or automatic cell counter to calculate the total number of Platelets per ml blood collected in Heparin or EDTA treated tubes.
6. Dilute the counted Platelets with PBS-EDTA 5 mM to a final concentration of $10x10^6$ cells/ml.

-B- Immuno-fluorescent Staining

7. Determine the needed amount of tubes (negative control (isotype control), positive control (CD61), CD55 and CD59 single or dual experiments).
8. Add 100 µl of leukocytes to each tube (2x106 cells).
9. Add 10 µl of the singles (CD55/CD59) or 20 µl of the dual.
10. Incubate for 30 min. at room temperature. Avoid direct light.
11. Wash twice in 3 ml PBS-EDTA 5 mM and centrifuge for 5 min. 2000g.
12. Resuspend the cells in PBS-EDTA 5 mM (200 – 500 µl).

-C- Flow Cytometry Data Acquisition

13. For FACS analysis, use a gate based on morphological parameters in order to eliminate cell debris and electronic background List mode files of 20.000 events should be collected for log FSC, log SSC and log fluorescence signals.

References

1. Socié G, Mary JY, de Gramont A, Rio B, Leporrier M, Rose C, et al. (1996) Paroxysmal nocturnal haemoglobinuria: long-term follow-up and prognostic factors. Lancet; 348:573-577.
2. Rotoli B, Luzzatto L (1989) Paroxysmal nocturnal haemoglobinuria. Bailliere's Clin Haematol; 2:113-138.
3. Kinoshita T, Inoue N, Takeda J (1995) Defective glycosyl phosphatidylinositol anchor synthesis and paroxysmal nocturnal hemoglobinuria. Adv Immunol; 60:57-103.
4. Yeh ETH, Rosse WF (1994) Paroxysmal nocturnal hemoglobinuria and the glycosylphosphatidylinositol anchor. J Clin Invest; 93:2305-2310.
5. van der Schoot CE, Huizinga TWJ, van't Veer-Korthof ET, Wijmans R, Pinkster J, von dem Borne AEGK (1990) Deficiency of glycosyl-phosphatidylinositol-linked membrane glycoproteins of leukocytes in paroxysmal nocturnal hemoglobinuria, description of a new diagnostic cytofluorometric assay. Blood; 76:1853-1859.
6. Navenot JM, Bernard D, Harousseau JL, Muller JY, Blanchard D (1996) Expression of glycosyl-phosphatidylinositol-linked glycoproteins in blood cells from paroxysmal nocturnal haemoglobinuria patients. A flow cytometry study using CD55, CD58 and CD59 monoclonal antibodies. Leuk Lymphoma; 21:143-151.
7. Hall SE, Rosse WF (1996) The use of monoclonal antibodies and flow cytometry in the diagnosis of paroxysmal nocturnal hemoglobinuria. Blood; 87:5332-5340.
8. Iwamoto N, Kawaguchi T, Nagakura S, Hidaka M, Horikawa K, Kagimoto T, et al. (1995) Markedly high population of affected reticulocytes negative for decay-accelerating factor and CD59 in paroxysmal nocturnal hemoglobinuria. Blood; 85:2228-2232.
9. Navenot JM (1996) Biological diagnosis and follow-up of paroxysmal nocturnal hemoglobinuria. Contribution to the study of physiopathology. Thesis for PhD, University of Nantes, France.

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.

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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