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AM26551FC-S Monoclonal Antibody to CD59 - FITC

Alternate names:	20 kDa homologous restriction factor, HRF-20, HRF20, MAC-IP, MAC-inhibitory protein, MACIF, MEM43 antigen, MIC11, MIN1, MIN2, MIN3, MIRL, MSK21, Membrane attack complex inhibition factor, Membrane inhibitor of reactive lysis, Protectin
Quantity:	0.1 ml
Concentration:	0.5 mg/ml
Background:	CD59, an 18-20 kDa GPI-anchored glycoprotein, is broadly distributed, present in most tissues, and on all circulating cells. CD59 has been also known as 20 kDa homologous restriction factor (HRF20), membrane attack complex inhibitory factor (MACIF), membrane inhibitor of reactive lysis (MIRL) and protectin. CD59 inhibits the cytolytic activity of complement by binding to C8 and C9 in the assembling of the cytolytic membrane attack complex (MAC), thereby interfering with membrane insertion and pore formation. CD59 and other membrane complement regulatory proteins (CD46 and CD55) are frequently overexpressed on cancer cells, possibly as a mechanism for cancer cells to overcome lysis by complement. CD59 also interacts with T cell-restricted glycoprotein CD2 and plays a role in T cell activation.
Uniprot ID:	P13987
NCBI:	NP_000602.1
GeneID:	966
Host / Isotype:	Mouse / IgG2b
Recommended Isotype Controls:	SM12F
Clone:	WK8
Immunogen:	Human myeloma cell line (RPMI8226)
Format:	**State:** Liquid Ig fraction **Purification:** Protein A agarose **Buffer System:** PBS containing 1% BSA and 0.09% NaN3 **Label:** FITC
Applications:	Flow cytometry: 10-20 µg/mL (final concentration). For details see protocols below. Other applications not tested. Optimal dilutions are dependent on conditions and should be determined by the user.
Specificity:	This antibody reacts with human CD59 antigen.
Add. Information:	This product was originally produced by MBL International.
Storage:	Store at 2-8 °C. Shelf life: one year from despatch.
General Readings:	1. Davies, A., et al., J. Exp. Med. 170, 637-654 (1989). 2. Okada, N., et al., Int. Immunol. 1, 205-208 (1989).

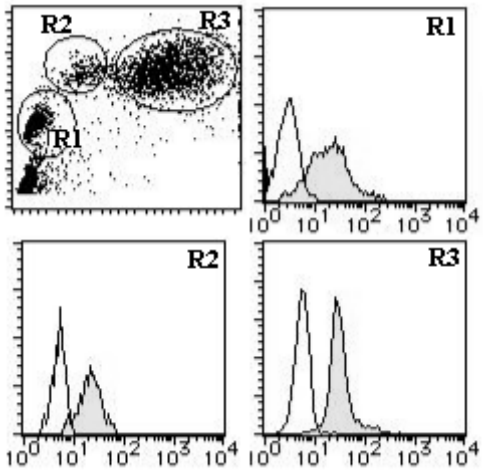
Protocols:

Flow cytometric analysis for whole blood cells
We usually use Falcon tubes or equivalents as reaction tubes for all steps described below.
1) Add 20 µL of the primary antibody at the concentration as suggest in the APPLICATIONS diluted with the washing buffer [PBS containing 2% fetal calf serum (FCS) and 0.1% NaN3] into each tube.
2) Add 100 µL of whole blood into each tube. Mix well, and incubate for 30 minutes at room temperature (20~25oC).
3) Add 1 mL of washing buffer followed by centrifugation at 500 x g for 1 minute at room temperature. Remove supernatant by careful aspiration.
4) Lyse with OptiLyse C (for analysis on Beckman Coulter instruments) or OptiLyse B (for analysis on BD instruments), using the procedure recommended in the respective package inserts.
5) Add 1 mL of H2O to each tube and incubate for 10 minutes at room temperature.
6) Centrifuge at 500 x g for 1 minute at room temperature. Remove supernatant by careful aspiration.
7) Add 1 mL of washing buffer followed by centrifugation at 500 x g for 1 minute at room temperature. Remove supernatant by careful aspiration.
8) Resuspend the cells with 500 µ L of the washing buffer and analyze by a flow cytometer.

Flow cytometric analysis for floating cells
We usually use Fisher tubes or equivalents as reaction tubes for all steps described below.
1) Wash the cells 3 times with washing buffer [PBS containing 2% fetal calf serum (FCS) and 0.1% NaN3].
2) Resuspend the cells with washing buffer (5 x 10e6 cells/mL).
3) Add 50 µL of the cell suspension into each tube, and centrifuge at 500 x g for 1 minute at room temperature (20~25oC). Remove supernatant by careful aspiration.
4) Add 20 µL of human Fc receptor blocking reagent to the cell pellet after tapping. Mix well and incubate for 5 minutes at room temperature.
5) Add 20 µL of the primary antibody at the concentration as suggest in the APPLICATIONS diluted in the washing buffer. Mix well and incubate for 30 minutes at room temperature.
6) Add 1 mL of the washing buffer followed by centrifugation at 500 x g for 1 minute at room temperature. Remove supernatant by careful aspiration.
7) Resuspend the cells with 500 µL of the washing buffer and analyze by a flow cytometer.
(Positive controls for Flow cytometry; HPB-ALL, HL-60)

Pictures:

Flow cytometric analysis of CD59 expression on human lymphocyte (R1), monocyte (R2) and granulocyte (R3) using AM26551FC-S. Open histograms indicate the reaction of isotypic control to the cells. Shaded histograms indicate the reaction of AM26551FC-S to the cells.



Flow cytometric analysis of CD59 expression on HPB-ALL cells (left) and HL-60 cells (right). Open histograms indicate the reaction of isotypic control to the cells. Shaded histograms indicate the reaction of AM26551FC-S to the cells.