For Research Use Only.
Not for use in diagnostic procedures.

MBL
A JSR Life Sciences Company

MONOCLONAL ANTIBODY

Anti-Granulysin (Human) mAb-Biotin

Code No.	Clone	Subclass	Quantity	Concentration
D185-6	RC8	Mouse IgG1 κ	100 µL	100 µg/mL

BACKGROUND: Granulysin is an antimicrobial protein expressed on cytotoxic T cells, natural killer (NK) cells and NKT cells. It has been shown that Granulysin contributes to the defence mechanisms against variety of mycobacterial infection and tumors. Granulysin has two molecular forms, 15 kDa precursor and 9 kDa effector form. Their serum levels were significantly elevated during the acute viral infections and correlated with the NK cell and CTL activities in patients with sever immunodeficiency, indicate that serum Granulysin could be useful novel marker to evaluate the overall status of host cell immunity.

SOURCE: This antibody was purified from hybridoma (clone RC8) supernatant using protein A agarose. This hybridoma was established by fusion of mouse myeloma cell Sp2/0-Ag8 with Balb/c mouse splenocyte immunized with the full-length human Granulysin expression plasmid.

FORMULATION: 10 µg IgG in 100 µL volume of PBS containing 1% BSA and 0.1% ProClin 150.

STORAGE: This antibody solution is stable for one year from the date of purchase when stored at 4°C.

REACTIVITY: This antibody is available for Granulysin ELISA.

APPLICATION:
ELISA; 1:1,000
*Please refer to the data sheet (MBL; code no. D185-3) for other applications.

Detailed procedure is provided in the following **PROTOCOL**.

INTENDED USE:
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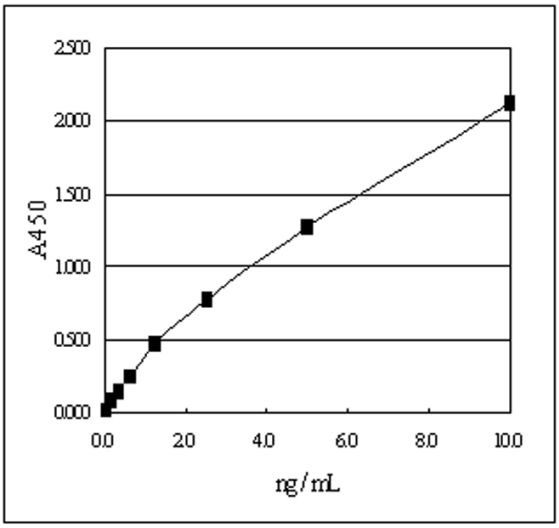
SPECIES CROSS REACTIVITY:

Species	Human	Mouse	Rat
Protein	recombinant	Not tested	Not tested
Reactivity on ELISA	+		

REFERENCES:
1) Pitabut, N., *et al*., *Int. J. Med. Sci.* **10**, 1003-1014 (2013) [ELISA]
2) Pitabut, N., *et al*., *Microbiol. Immunol.* **55**, 565-573 (2011) [ELISA]
3) Nakashima, A., *et al*., *Am. J. Pathol*. **173**, 653-664 (2008)
4) Ogawa, K., *et al*., *Eur. J. Immunol*. **33**, 1925-1933 (2003)
5) Gamen, S., *et al*., *J. Immunol*. **161**, 1758-1764 (1998)

Clone RC8 is used in reference number 1) - 4).

The descriptions of the following protocols are examples. Each user should determine the appropriate condition.



Granulysin ELISA analysis. *Plates are coated with the capture antibody (D184-3) and are added the recombinant human Granulysin and are measured by the biotinylated detector antibody (D185-6).*

PROTOCOL:
ELISA
A. The preparation of the microtiter plate
1) Distribute 100 µL/well of 2 µg/mL Anti-Granulysin (Human) mAb (D184-3) diluted with PBS to the 96-well microtiter plate.
2) Incubate at 4°C overnight.
3) Distribute 200 µL/well of the blocking buffer (5% sucrose, 1% BSA, 0.1% NaN_3 in PBS).
4) Incubate at 4°C overnight.
5) Discard the blocking buffer and dry up.

B. ELISA assay

6) Distribute 100 µL/well of the recombinant Granulysin standard (0, 0.16, 0.31, 0.63, 1.25, 2.50, 5.00, 10.00 ng/mL) diluted with the blocking buffer to each well.
7) Incubate it for 1 hour at room temperature.
8) Wash the plates 4 times with 200 µL/well of PBS-T [0.05% Tween-20 in PBS].
9) Distribute 100 µL/well of 1:1,000 Anti-Granulysin (Human) mAb-Biotin (D185-6) diluted with the blocking buffer to each well.
10) Incubate it for 1 hour at room temperature.
11) Wash the plates 4 times with 200 µL/well of PBS-T.
12) Distribute 100 µL/well of 20 ng/mL the HRP-conjugated streptavidin diluted with PBS-T to each well.
13) Incubate it for 1 hour at room temperature.
14) Wash the plates 4 times with 200 µL/well of PBS-T.
15) Distribute 100 µL/well of the substrate solution (tetramethyl benzidine solution).
16) Incubate at room temperature for 30 minutes.
17) Distribute 100 µL/well of 0.5 M H_2SO_4 to each well and stop enzyme reaction.
18) After gentle mixing, determine the absorbance at 450 nm of each well by a spectrophotometer.

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