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Not for use in diagnostic procedures.

MBL
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MONOCLONAL ANTIBODY

Anti-Granulysin (Human) mAb

Code No.	Clone	Subclass	Quantity	Concentration
D184-3	RB1	Mouse IgG1 κ	100 µL	1 mg/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 defense mechanisms against variety of mycobacterial infection and tumors. Granulysin has two molecular forms, 15-kDa precursor and 9-kDa effector form. There 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 RB1) 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 expressing plasmid.

FORMULATION: 100 µg IgG in 100 µL volume of PBS containing 50% glycerol, pH 7.2. No preservative is contained.

STORAGE: This antibody solution is stable for one year from the date of purchase when stored at -20°C.

REACTIVITY: This antibody reacts with human Granulysin on Immunoprecipitation, Immunocytochemistry and Flow cytometry.

APPLICATIONS:

Western blotting; Not recommended
Immunoprecipitation; 10 µg/200 µL of cell extract from $5x10^6$ cells
Immunohistochemistry; Not tested
Immunocytochemistry; 10 µg/mL
Flow cytometry; 10 µg/mL (final concentration)
ELISA; Not tested*
*It is reported that this monoclonal antibody can be used in ELISA in the reference number 1) and 2).

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

INTENDED USE:

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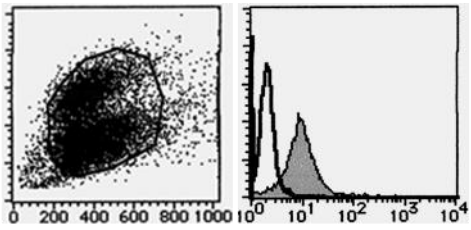
SPECIES CROSS REACTIVITY:

Species	Human	Mouse	Rat
Cells	PBMC, YT	Not tested	Not tested
Reactivity on IP	+		

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) Fujita, Y., *et al., J. Am. Acad. Dermatol.* **65**, 65-68 (2011)
4) Legrand, F., *et al., J. Immunol.* 185, 7443-7451 (2010) [FCM]
5) Ogawa, K., *et al., Eur. J. Immunol.***33**, 1925-1933 (2003)
6) Gamen, S., *et al., J. Immunol.* **161**, 1758-1764 (1998)

Clone RB1 is used in reference number 1)-5).



Flow cytometric analysis of Granulysin expression in YT cells. *Open histogram indicates the reaction of Isotypic control to the cells. Shaded histogram indicates the reaction of D184-3 to the cells.*

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

PROTOCOLS:

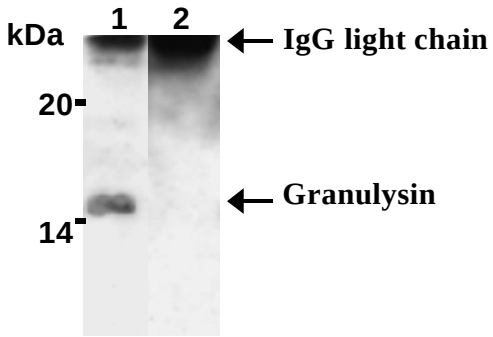
Flow cytometric analysis for floating cells

We usually use Fisher tubes or equivalents as reaction tubes for all step described below.

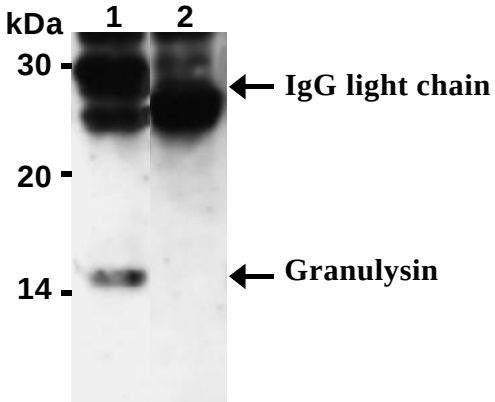
1) Wash the cells 3 times with washing buffer [PBS containing 2% fetal calf serum (FCS) and 0.1% NaN_3].
*Azide may react with copper or lead in plumbing system to form explosive metal azides. Therefore, always flush plenty of water when disposing materials containing azide into drain.
2) Add 200 µL of 4% paraformaldehyde (PFA) to the cell pellet after tapping. Mix well, and then fix the cells for 15 minutes at 4°C.
3) Wash the cells 3 times with the washing buffer.
4) Add 200 µL of 70% ethanol to the cell pellet after tapping. Mix well and permeabilize the cells for 30 minutes at -20°C.

5) Wash the cells 3 times with the washing buffer.
6) Add 10 µL of Clear Back (human Fc receptor blocking reagent, MBL; code no. MTG-001) to the cell pellet after tapping. Mix well and incubate for 5 minutes at room temperature (20~25°C).
7) Add 40 µL of the primary antibody at the concentration of as suggested in the **APPLICATIONS** diluted with the washing buffer. Mix well and incubate for 30 minutes at room temperature.
8) 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.
9) Add 30 µL of 1:40 Anti-IgG (Mouse) pAb-FITC (Beckman Coulter; code no. IM0819) diluted with the washing buffer. Mix well and incubate for 15 minutes at room temperature.
10) 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.
11) Resuspend the cells with 500 µL of the washing buffer and analyze by a flow cytometer.

(Positive control for Flow cytometry; YT)



Immunoprecipitation of Granulysin from PBMC with D184-3 (1) or mouse IgG1(2). *After immunoprecipitated with the antibody, immunocomplex was resolved on SDS-PAGE and immunoblotted with D186-3.*



Immunoprecipitation of Granulysin from YT cells with D184-3 (1) or mouse IgG1 (2). *After immunoprecipitated with the antibody, immunocomplex was resolved on SDS-PAGE and immunoblotted with D186-3.*

Immunoprecipitation

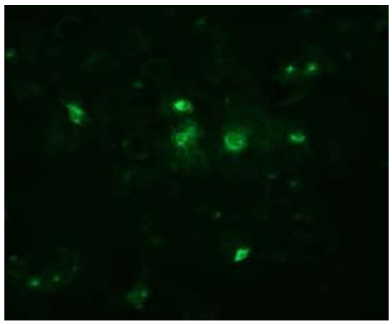
1) Wash the cells 3 times with PBS and suspend with 10 volumes of cold Lysis buffer [50 mM Tris-HCl (pH 7.2), 250 mM NaCl, 0.1% NP-40, 2 mM EDTA, 10% glycerol] containing appropriate protease inhibitors. Incubate it at 4°C with rotating for 30 minutes, then sonicate briefly (up to 10 seconds).
2) Centrifuge the tube at 12,000 x g for 10 minutes at 4°C and transfer the supernatant to another tube.
3) Add primary antibody as suggested in the **APPLICATIONS** into 200 µL of the supernatant. Mix well and incubate with gentle agitation for 30-120 minutes at 4°C. Add 20 µL of 50% protein A agarose beads resuspended in the cold Lysis buffer. Mix well and incubate with gentle agitation for 60 minutes at 4°C.
4) Centrifuge the tube at 2,500 x g for 10 seconds and discard the supernatant.
5) Resuspend the agarose with cold Lysis buffer.
6) Centrifuge the tube at 2,500 x g for 10 seconds and discard the supernatant.
7) Repeat steps 5)-6) 2-4 times.
8) Resuspend the beads in 20 µL of Laemmli's sample buffer. Boil the samples for 2 minutes and centrifuge. Load 10 µL of the sample per lane in a 1 mm thick SDS-polyacrylamide gel for electrophoresis.
9) Blot the protein to a polyvinylidene difluoride (PVDF) membrane at 1 mA/cm^2 for 1 hour in a semi-dry transfer system (Transfer Buffer [25 mM Tris, 190 mM glycine, 20% Methanol]). See the manufacture's manual for precise transfer procedure.
10) To reduce nonspecific binding, soak the membrane in 10% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature, or overnight at 4°C.
11) Incubate the membrane with 10 µg/mL of Anti-Granulysin (Human) mAb (MBL; code no. D186-3) diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature. (The concentration of antibody will depend on the conditions.)
12) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 minutes x 3)
13) Incubate the membrane with Anti-IgG (H+L chain) (Mouse) pAb-HRP (MBL; code no. 330) diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature.
14) Wash the membrane with PBS-T (5 minutes x 6).
15) Wipe excess buffer on the membrane, then incubate it with appropriate chemiluminescence reagent for 1 minute. Remove extra reagent from the membrane by dabbing with paper towel, and seal it in plastic wrap.
16) Expose to an X-ray film in a dark room for 5 minutes. Develop the film as usual. The condition for exposure and development may vary.

(Positive controls for Immunoprecipitation; PBMC and YT)

Immunocytochemistry

1) Culture the cells in the appropriate condition on a glass slide. (For example, spread 1 x 10^4 cells of Granulysin transfected COS7 cells for one slide, and then incubate in a CO_2 incubator for one night.)
2) Fix the cells by immersing the slide in PBS containing 2% paraformaldehyde for 20 minutes at room temperature.
3) The glass slide was washed with PBS 3 times.
4) Immerse the slide in PBS containing 0.5% Tween-20 for 15 minutes at room temperature.
5) The glass slide was washed with PBS 3 times.
6) Add the primary antibody diluted with PBS containing 5% normal goat serum as suggested in the **APPLICATIONS** onto the cells and incubate for 30 minutes at room temperature. (Optimization of antibody concentration or incubation condition is recommended if necessary.)
7) The glass slide was washed with PBS 3 times.
8) Add 50 µL of 1:40 Anti-IgG (Mouse) pAb-FITC (Beckman Coulter; code no. IM0819) diluted with PBS containing 5% normal goat serum onto the cells. Incubate for 20 minutes at room temperature. Keep out light by aluminum foil.
9) The glass slide was washed with PBS 3 times.
10) Wipe excess liquid from slide but take care not to touch the cells. Never leave the cells to dry.
11) Promptly add mounting medium onto the slide, then put a cover slip on it.

(Positive control for Immunocytochemistry; Transfectant)



Immunocytochemical detection of Granulysin in 2% PFA fixed Granulysin transfected COS7 cells with D184-3.

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