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

MBL A JSR Life Sciences Company

MONOCLONAL ANTIBODY

Anti-GPI-80 (Human) mAb

Code No.	Clone	Subclass	Quantity	Concentration
D087-3	3H9	Mouse IgG1	100 µL	1 mg/mL

BACKGROUND: The GPI-80 molecule (80 kDa) recognized by this antibody (clone 3H9) was shown to be present on human neutrophils. When 3H9 was added with a neutrophil stimulant (fMLP), the inhibition of neutrophil adherence was observed after 60 minutes incubation. 3H9 enhanced not only fMLP-induced chemotaxis but random migration of neutrophil as well. Furthermore, 3H9 clearly discriminated neutrophils from both basophils and eosinophils derived from humans.

SOURCE: This antibody was purified from hybridoma (clone 3H9) supernatant using protein A agarose. This hybridoma was established by fusion of mouse myeloma cell X63-Ag8.653 with Balb/c mouse splenocyte immunized with PMA activated human neutrophil.

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 GPI-80 on Western blotting and Flow cytometry.

APPLICATIONS:
Western blotting; 1-5 µg/mL
Immunoprecipitation; Not tested
Immunohistochemistry; Not tested
Immunocytochemistry; Not tested
Flow cytometry; 1-10 µg/mL (final concentration)

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

SPECIES CROSS REACTIVITY:

Species	Human	Mouse	Rat	Others
Cells	Granulocyte	Neutrophil	Neutrophil	Not tested
Reactivity on WB	+	-	-	

INTENDED USE:
For Research Use Only. Not for use in diagnostic procedures.

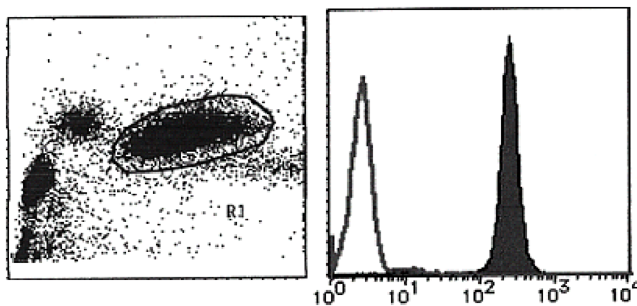
REFERENCES:

1) Takeda, Y., *et al., Clin. Exp. Immunol.* **186**, 373-386 (2016) [FCM]
2) Sendo, D., *et al., Yamagata Med. J.* **23**, 69-82 (2005)
3) Yoshitake, H., *et al., J. Leukoc. Biol.* **71**, 205-211 (2002)
4) Dahlgren, C., *et al. J. Leukoc. Biol.* **69**, 57-62 (2001)
5) Huang, J., *et al. Microbiol. Immunol.* **45**, 467-71 (2001)
6) Nakamura-Sato, Y., *et al., J. Leukoc. Biol.*, **68**, 650-654 (2000)
7) Suzuki, K., *et al. J. Immunol.* **162**, 4277-84 (1999)
8) Ohtake, K., *et al., Microbiol. Immunol.* **41**, 67-72 (1997)

Clone 3H9 is used in these references.

RELATED PRODUCT:
Please visit our website at https://ruo.mbl.co.jp/.

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



Flow cytometric analysis of human GPI-80 expression on human granulocyte. *Open histogram indicates the reaction of isotypic control to the cells. Shaded histogram indicates the reaction of D087-3 to the cells.*

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 0.09% 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) Resuspend the cells with washing buffer ($5x10^6$ 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 (20~25°C). Remove supernatant by careful aspiration.

4) Add 10 µL of normal goat serum containing 1 mg/mL normal human IgG and 0.09% NaN_3 to the cell pellet after tapping. Mix well and incubate for 5 minutes at room temperature.
5) Add 40 µL primary antibodies at the concentration of as suggest in the **APPLICATIONS** diluted with 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) Add FITC conjugated anti-mouse IgG antibody diluted with the washing buffer. Mix well and incubate for 15 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) Resuspend the cells with 500 µL of the washing buffer and analyze by a flow cytometer.

Flow cytometric analysis for whole blood cells

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

1) Add 50 µL of c diluted with the washing buffer [PBS containing 2% fetal calf serum (FCS) and 0.09% NaN_3] into each tube.
2) Add 50 µL of whole blood into each tube. Mix well, and incubate for 30 minutes at room temperature (20~25°C).
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) Add FITC conjugated anti-mouse IgG antibody diluted with washing buffer. Mix well and incubate for 15 minutes at room temperature.
5) Add 1 mL of washing buffer followed by centrifugation at 500 x g for 1 minute at room temperature. Remove supernatant by careful aspiration.
6) 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.
7) Add 1 mL of H_2O to each tube and incubate for 10 minutes at room temperature.
8) Centrifuge at 500 x g for 1 minute at room temperature. Remove supernatant by careful aspiration.
9) Add 1 mL of washing buffer followed by centrifugation at 500 x g for 1 minute at room temperature. Remove supernatant by careful aspiration.
10) Resuspend the cells with 500 µL of the washing buffer and analyze by a flow cytometer.

SDS-PAGE & Western Blotting

1) Preparation of the granulocytes from whole blood.
   1. Put 5 mL of EDTA treated whole blood onto the 5 mL of HistoPAQUE-1077 (SIGMA) in a 15 mL Fisher tube.
   2. Centrifuge at 16,000 rpm for 30 minutes at room temperature.
   3. Aspirate 'plasma-layer', 'lymphocyte-layer' and 'Ficoll-layer' carefully.
   4. Add PBS to the tube (same volume of the residual erythrocyte/leukocyte layer) and mix gently.
   5. Centrifuge at 16,000 rpm for 30 minutes at 4°C.
   6. Remove the supernatant, add same volume of PBS and transfer the contents to a 50 mL tube.
   7. Add 2.5 mL of OptiLyse B (for analysis on BD instruments) or OptiLyse C (for analysis on Beckman Coulter instruments). Mix the sample gently and incubate for 10 minutes at room temperature.
   8. Add 50 mL of distilled water into the tube. Mix the sample gently and incubate for 10 minutes at room temperature followed by centrifugation at 3,000 rpm for 5 minutes at 4°C. Remove the supernatant by careful aspiration.
   9. Resuspend the cells with appropriate volume of PBS and check the number of leukocytes (about $3x10^7$ cells).
   10. After collecting the cells by centrifugation, resuspend the cells with 100 µL of PBS containing 1% TrironX-100, 1 mM PMSF and 10% glycerol, then incubate for 60 minutes at room temperature.
   11. Transfer the content to a 1.5 mL tube and centrifuge at 600 x g for 30 minutes at 4°C.
   12. Transfer the supernatant to another tube and centrifuge at 14,000 x g for 15 minutes at 4°C. Use the supernatant as sample.
2) Centrifuge the tube at 12,000 x g for 10 minutes at 4°C and transfer the supernatant to another tube. Measure the protein concentration of the supernatant and add the cold Lysis buffer to make 8 mg/mL solution.
3) Mix the sample with equal volume of Laemmli's sample buffer.
4) Boil the samples for 3 minutes and centrifuge. Load 10 µL of the sample per lane in a 1 mm thick SDS-polyacrylamide gel for electrophoresis.
5) 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% MeOH). See the manufacture's manual for precise transfer procedure.
6) 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.
7) Incubate the membrane with primary antibody diluted with PBS, pH 7.2 containing 1% skimmed milk as suggest in the **APPLICATIONS** for 1 hour at room temperature. (The concentration of antibody will depend on condition.)
8) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 minutes x 3 times).
9) Incubate the membrane with 1:10,000 Anti-IgG (Mouse) pAb-HRP (MBL; code no. 330) diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hour at room temperature.
10) Wash the membrane with PBS-T (10 minutes x 3 times).
11) Wipe excess buffer on the membrane, then incubate it with appropriate chemiluminescence reagent for 1 minute.
12) Remove extra reagent from the membrane by dabbing with paper towel, and seal it in plastic wrap.

13) Expose to an X-ray film in a dark room for 3 minutes. Develop the film as usual. The condition for exposure and development may vary.

    (Positive control for Western blotting; Granulocyte)