

MONOCLONAL ANTIBODY

Anti-GPI-80 (Human) mAb-PE

Code No.	Clone	Subclass	Quantity
D087-5	3H9	Mouse IgG1	1 mL (50 tests)

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 neutrophils 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: 50 tests in 1 mL volume of PBS containing 1% BSA and 0.09% NaN₃.

*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.

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

REACTIVITY: This antibody reacts with human GPI-80 on flow cytometry.

APPLICATIONS:

Western blotting; Not tested

Immunoprecipitation; Not tested

Immunohistochemistry; Not tested

Immunocytochemistry; Not tested

Flow Cytometry; 20 µL (ready for use)

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

SPECIES CROSS REACTIVITY:

Species	Human	Mouse	Rat
Cells	Granulocyte	Nneutrophil	Neutrophil
Reactivity on FCM	+	-	-

INTENDED USE:

For research use only. Not for use in diagnostic procedures.

REFERENCES:

- 1) Sumide, K., *et al. Nat. Commun.* **9**, 2202 (2018) [FCM]
- 2) Ng, E. S., *et al. Nat. Biotechnol.* **34**, 1168-1179 (2016) [FCM]
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- 4) Takeda, Y., *et al. Clin. Exp. Immunol.* **186**, 373-386 (2016) [FCM]
- 5) Saeki, K., *et al., Stem Cells* **27**, 59-67 (2009) [FCM]
- 6) Sendo, D., *et al., Yamagata Med. J.* **23**, 69-82 (2005)
- 7) Yoshitake, H., *et al., J. Leukoc. Biol.* **71**, 205-211 (2002)
- 8) Dahlgren, C., *et al. J. Leukoc. Biol.* **69**, 57-62 (2001)
- 9) Huang, J., *et al. Microbiol. Immunol.* **45**, 467-471 (2001)
- 10) Nakamura-Sato, Y., *et al., J. Leukoc. Biol.*, **68**, 650-654 (2000)
- 11) Suzuki, K., *et al. J. Immunol.* **162**, 4277-84 (1999)
- 12) Ohtake, K., *et al., Microbiol. Immunol.* **41**, 67-72 (1997)

Clone 3H9 is used in these references.

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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.

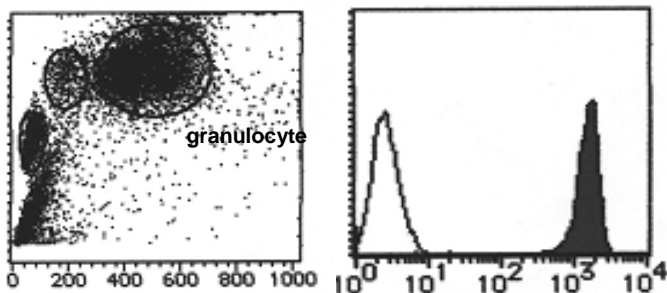
- 1) Wash the cells 3 times with washing buffer [PBS containing 2% fetal calf serum (FCS) and 0.09% NaN₃].
*Azide may react with copper or lead in plumbing system to form explosive metal azides. Therefore, always flush plenty of water when disposing materials containing azide into drain.
- 2) Resuspend the cells with washing buffer (5 x 10⁶ 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 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.
- 5) Add the primary antibody as suggested in the **APPLICATIONS**. 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.

Flow cytometric analysis for whole blood cells

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

- 1) Add the primary antibody as suggested in the **APPLICATIONS** into each tube.
- 2) Add 100 μ L 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 [PBS containing 2% fetal calf serum (FCS) and 0.09% NaN₃] 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.

(Positive control for flow cytometry: Granulocyte)



Flow cytometric analysis of Human GPI-80 expression on Granulocyte. Open histogram indicates the reaction of isotypic control to the cells. Shaded histogram indicates the reaction of D087-5 to the cells.