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Not for use in diagnostic procedures.

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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 0.1% ProClin 150.

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

REACTIVITY: This antibody 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:
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REFERENCES:
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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]
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9) Huang, J., *et al. Microbiol. Immunol.* **45**, 467-471 (2001)
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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.

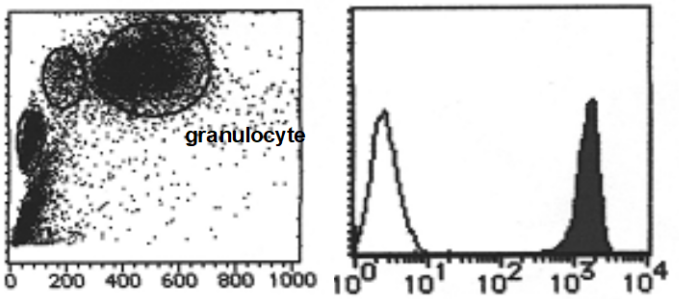
PROTOCOLS:
Flow cytometric analysis for floating cells
We usually use Fisher tubes or equivalents as reaction tubes for all step described below.
1) Wash the cells 3 times with washing buffer [PBS containing 2% fetal calf serum (FCS) and 0.09% NaN_3].
   *Azide may react with copper or lead in plumbing 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 (5 x 10^6 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 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 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 [PBS containing 2% fetal calf serum (FCS) and 0.09% NaN_3] 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 H_2O 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 µL 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.*