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Product datasheet for AM26453AF-N

VNN2 Mouse Monoclonal Antibody [Clone ID: 3H9]

Product data:

Product Type:	Primary Antibodies
Clone Name:	3H9
Applications:	FC, WB
Recommended Dilution:	**Western blot:** 1-5 µg/ml for chemiluminescence detection system. **Flow cytometry:** 1-10 µg/ml (final concentration). For details see protocols below.
Reactivity:	Human
Host:	Mouse
Isotype:	IgG1
Clonality:	Monoclonal
Immunogen:	PMA activated human neutrophil
Specificity:	This antibody reacts with human GPI-80. Does not react with mouse or rat Neutrophil.
Formulation:	PBS containing 50% glycerol, pH 7.2. No preservative is contained. State: Azide Free State: Liquid Ig fraction
Concentration:	lot specific
Purification:	Protein A agarose
Conjugation:	Unconjugated
Storage:	Store (in aliquots) at -20 °C. Avoid repeated freezing and thawing.
Stability:	Shelf life: one year from despatch.
Gene Name:	vanin 2
Database Link:	Entrez Gene 8875 Human O95498

View online »

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.

Synonyms: VNN2

Note: This product was originally produced by MBL International.

Protocol:

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% NaN3].
2) Resuspend the cells with washing buffer (5x10e6 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 (20~25oC). Remove supernatant by careful aspiration.
4) Add 10 µL of normal goat serum containing 1 mg/mL normal human IgG and 0.1% NaN3 to the cell pellet after tapping. Mix well and incubate for 5 minutes at room temperature.
5) Add 40 µL primary antibody 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 30 µL of 1:100 FITC conjugated anti-mouse IgG 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.

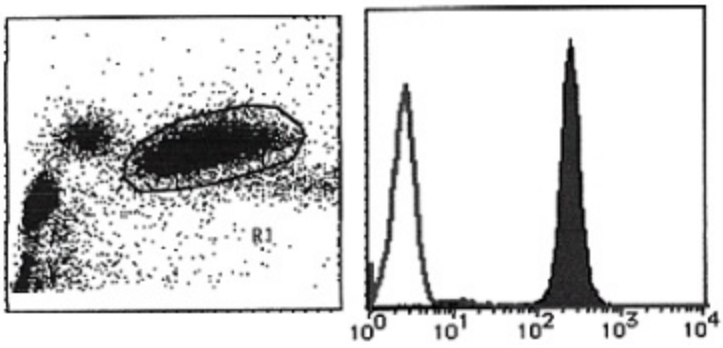
Flow cytometric analysis for whole blood cells

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 0.1% NaN3] into each tube.
2) Add 50 µL of whole blood into each tube. Mix well, and incubate for 30 minutes at room temperature (20~25oC).
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 30 µL of 1:100 FITC conjugated anti-mouse IgG 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 H2O 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 4oC.
6.Remove the supernatant, add same volume of PBS
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 4oC. Remove the supernatant by careful aspiration.
9. Resuspend the cells with appropriate volume of PBS and check the number of leukocytes (about 3x10e7 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 4oC.
12. Transfer the supernatant to another tube and centrifuge at 14,000 x g for 15 minutes at 4oC. Use the supernatant as sample.
2) Centrifuge the tube at 12,000 x g for 10 minutes at 4oC 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/cm2 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 4oC.
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 the 1:10,000 HRP-conjugated anti-mouse IgG 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)

Product images:



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 AM26453AF-N to the cells.