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

MONOCLONAL ANTIBODY

Anti-NEU3 (Human) mAb

Code No.	Clone	Subclass	Quantity	Concentration
D164-3	11B	Mouse IgG1 κ	100 µL	1 mg/mL

BACKGROUND: Gangliosides, sialic acid-containing glycosphingolipids, are present in surface membranes of cells and play important functional roles in regulating a wide range of biological processes including cell surface interactions, cell differentiation, and transmembrane signaling. Plasma membrane-associated ganglioside sialidase (NEU3) has been suggested to play essential roles in regulation of cell surface functions because of its major localization in the plasma membrane and strict substrate preference for gangliosides, thought to play important roles in cell surface events through modulation of gangliosides.

SOURCE: This antibody was purified from hybridoma (clone 11B) supernatant using protein A agarose. This hybridoma was established by fusion of mouse myeloma cell P3-X63-Ag8.653 with Balb/c mouse splenocyte immunized with the human NEU3 transfected COS cells.

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 NEU3 (48 kDa) on Western blotting.

APPLICATIONS:

Western blotting; 1 µg/mL
Immunoprecipitation; Not tested*
*It is reported that this clone can be used in this application in the reference number 5) and 6).
Immunohistochemistry; Not tested*
*It is reported that this clone can be used in this application in the reference number 3).
Immunocytochemistry; Not tested*
*It is reported that this clone can be used in this application in the reference number 1).
Flow cytometry; Not tested

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

INTENDED USE:
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SPECIES CROSS REACTIVITY:

Species	Human	Mouse	Rat
Cells	Transfectant	Not tested	Not tested
Reactivity on WB	+		

REFERENCES:

1) Wang, J., *et al., J. Neurochem.* **111**, 547-554 (2009) [WB, IC]
2) Gadhoum, S. Z and Sackstein, R., *Nat. Chem. Biol.* **4**, 751-757 (2008) [WB]
3) Nomura, H., *et al., Oncol. Res.* **16**, 289-297 (2006) [IHC]
4) Wada, T., *et al., Oncogene* **26**, 2483-2490 (2007)
5) Sasaki, A., *et al., J. Biol. Chem.* **278,** 27896-27902 (2003)
6) Wang, Y., *et al., J. Biol. Chem.* **277,** 26252-26259 (2002)

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

PROTOCOL:

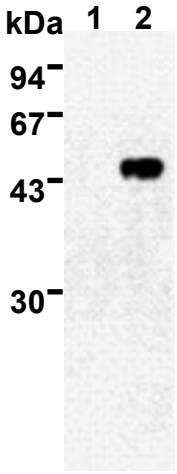
SDS-PAGE & Western blotting

1) Wash the cells 3 times with PBS and suspend with 10 volume 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. 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 2 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% Methanol). See the manufacturer'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 suggested in the **APPLICATIONS** for 1 hour at room

temperature. (The concentration of antibody will depend on the conditions.)

8) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 minutes x 6 times).
9) Incubate the membrane with the 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 (5 minutes x 6 times).
11) Wipe excess buffer on the membrane, and 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 10 minutes.
14) Develop the film as usual. The condition for exposure and development may vary.

(Positive control for Western blotting; Transfectant)



Western blot analysis of NEU3
Lane 1: Parental cell (COS-1)
Lane 2: Transfectant (NEU3-COS-1)
Immunoblotted with D164-3

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