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

MONOCLONAL ANTIBODY

Anti-p75NTR (Mouse) mAb

Code No.	Clone	Subclass	Quantity	Concentration
D297-3	25-8	Rat IgG2a	100 µL	1 mg/mL

BACKGROUND: p75NTR (p75 neurotrophin receptor) as known as LNGFR/p75NGFR/TNFRSF16/NGFR, a member of the TNF receptor superfamily, is a low affinity nerve growth factor receptor. Expression of p75NTR is observed in a number of non-neuronal and neuronal cells including motor neurons during development and also in damaged neurons. It is known that p75NTR/NGF signal transduction involved in both cell survival as well as cell death of neural cells and apoptosis induction of neurons.

SOURCE: This antibody was purified from hybridoma (clone 25-8) supernatant using protein G agarose. This hybridoma was established by fusion of mouse myeloma cell P3X with Wister rat lymphocyte immunized with mouse fetal hepatic 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 mouse p75NTR on Flow cytometry.

APPLICATIONS:

Flow cytometry; 10 µg/mL (final concentration)
Western blotting; Not tested
Immunoprecipitation; Not tested
Immunohistochemistry; Not tested
Immunocytochemistry; Not tested

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

INTENDED USE:

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REFERENCE:

1) Suzuki, K., *et al.*, *Gastroenterology* **135**, 270-281 (2008)

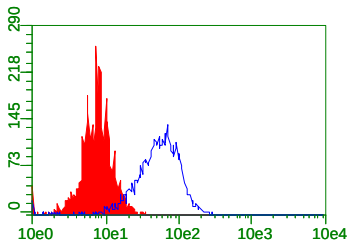
Clone 25-8 is used in this reference.

SPECIES CROSS REACTIVITY:

Species	Human	Mouse	Rat
Cell	Not tested	Transfectant	Not tested
Reactivity on FCM		+	

RELATED PRODUCT:

M081-3 Rat IgG2a (isotype control) (2H3)



Flow cytometric analysis of mouse p75NTR expressed in transfectant. *Shaded histogram indicates the reaction of isotypic control to the cells. Open histogram indicates the reaction of D297-3 to the cells.*

PROTOCOL:

Flow cytometric analysis for floating cells

We usually use Fisher tubes or equivalents as reaction tubes for all steps 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 100 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 20 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 20 µL of the primary antibody at the concentration as suggested in the **APPLICATIONS** diluted in 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.

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Remove supernatant by careful aspiration.

7) Add PE conjugated anti-rat 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.

(Positive control for Flow cytometry; Transfectant)