

MONOCLONAL ANTIBODY

Anti-mouse p75NTR

Code No.	Clone	Subclass	Quantity	Concentration
D297-3	25-8	Rat IgG2a	100 μ L	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 μ g IgG in 100 μ L 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 μ g/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:

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

REFERENCE:

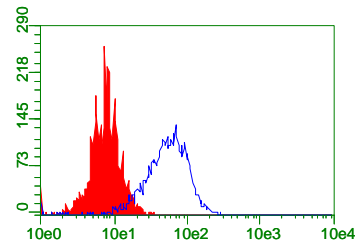
- 1) Suzuki, K., *et al.*, *Gastroenterology* **135**, 270-281 (2008)
Clone 25-8 is used in this reference.

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 0.1% NaN_3].
- 2) Resuspend the cells with washing buffer (5×10^6 cells/mL).
- 3) Add 100 μ L of the cell suspension into each tube, and centrifuge at 500 x g for 1 minute at room temperature ($20 \sim 25^{\circ}\text{C}$). Remove supernatant by careful aspiration.
- 4) Add 20 μ L 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 μ L 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. Remove supernatant by careful aspiration.
- 7) Add 20 μ L of 1:100 PE conjugated anti-rat IgG (MBL; code no. IM-1623) 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 μ L of the washing buffer and analyze by a flow cytometer.