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PRODUCT DATASHEET

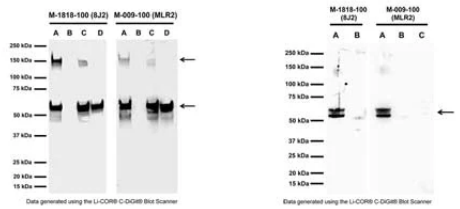
Anti-NGFR/p75 neurotrophin receptor (p75NTR) Antibody

Product Details

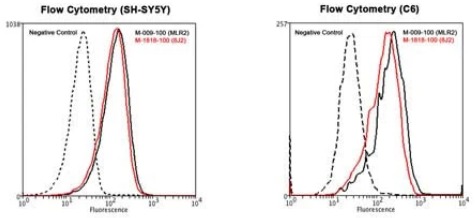
Available Variants	100 µg (SKU:M-1818-100)
Conjugate	Unconjugated
Isotype	IgG2a
Clone	8J2
Host Species	Mouse
Format	IgG
Physical State	Lyophilized
Buffer	Lyophilized from a solution containing PBS buffer pH 7.2-7.6 with 0.1% trehalose, without preservatives.
Production Notes	Protein A purified IgG
Applications	Flow, ICC, IHC, IP, WB
Dilution Ranges	WB: 0.5-2.0 µg/mL, **non-reducing conditions only** (no DTT or beta-mercaptoethanol). ICC: 1-5 µg/mL IP: Lysate dependent. 10 ug per 200-500 ug total protein
Species Reactivity	Human, Mouse, Rat
Immunogen	Recombinant extracellular domain (amino acids 29-250) of human NGFR/p75NTR protein with N-terminal His-tag.
Specificity	Reacts with human, mouse and rat. Cross-reactivity with other species not tested but expected.This antibody is specific for NGFR/p75NTR as demonstrated by Western blotting and immunprecipitation. The antibody recognizes extracellular p75NTR under non-reducing conditions.
Storage	Spin vial briefly before opening. Reconstitute in 100 µL sterile-filtered, ultrapure water to achieve a concentration of 1 mg/mL. Centrifuge to remove any

	insoluble material. Final buffer contains no preservatives. Store lyophilized antibody at 2-8°C. After reconstitution divide into aliquots and store at -20°C for long-term storage. Store at 2-8°C short-term (up to 4 weeks) with an appropriate antibacterial agent. Avoid repetitive freeze/thaw cycles.
UniProt ID	P08138
Country of Origin	United States
Shipping	25°C (ambient)
Expiration	12 months after date of receipt (unopened vial).
Usage Statement	For research use only.

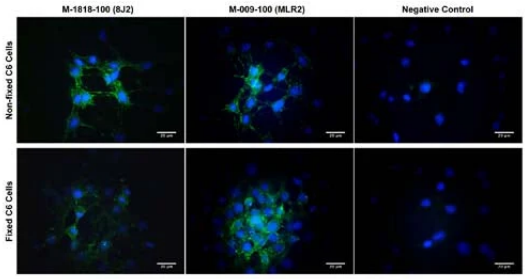
Product Images



Left: Analysis of p75NTR expression in human and rodent RIPA cell lysates by **Western Blotting**. Mouse antibody to p75NTR clone 8J2 (M-1818-100) and clone MLR2 (M-009-100) detect p75NTR-IR at ~50-60 kDa in mouse p75NTR-transfected cells (A), but not in non-transfected control cells (B). p75NTR-IR is also observed in human SH-SY5Y (C) and rat C6 (D) cell lysates. Dependent on cell lysate, dimers or trimers (~150 kDa) of p75NTR are detected (Anastasia et al., 2015). 50 ug of protein were loaded per lane. *WB Method*: SDS-PAGE: 4-12%, **non-reducing conditions**; Transfer: Tris-Glycine buffer; Membrane: nitrocellulose (0.45 um); Blocking: 5% skim milk in TBST, 1 hour at RT; Primary antibody: 1 µg/mL, overnight at 4°C; Secondary antibody: anti-mouse-HRP (1/6000) 1 hour at RT; Detection: Chemiluminiscence. **Right: Immunoprecipitation** of p75NTR from rat C6 cell lysate and detection by Western Blotting. Mouse antibody to p75NTR clone 8J2 (M-1818-100) and clone MLR2 (M-009-100) precipitate bands at the expected molecular weight of ~50-60 kDa for p75NTR (A). No p75NTR-IR is observed in remaining supernatant (B) or control (Protein G only, C). *IP Method:* C6 lysates were prepared in non-denaturing lysis buffer and pre-cleared with Protein G agarose beads (40 uL bead slurry per 1 mg total protein). Cleared lysate was then incubated with either M-1818-100 or M-009-100 (10 ug antibody per 200 ug total protein), and immune complexes bound by Protein G agarose (10 uL). Precipitated p75NTR was eluted off the beads and antibody by heating and addition of SDS-PAGE sample buffer. *WB Method*: as above, with primary goat anti-p75NTR (1 µg/mL) antibody and secondary anti-sheep-HRP (1/6000) antibody. Detection: Chemiluminiscence.



Binding analysis of M-1818-100 (8J2) and M-009-100 (MLR2) antibodies by **1-site ELISA** on human and mouse p75NTR protein. Clone 8J2 detects both, human and mouse p75NTR proteins with higher affinity than clone MLR2.



Analysis of membrane p75NTR expression in rat C6 cells by **Immunocytochemistry**. Non-permeabilized cells were stained with mouse antibodies to p75NTR clones 8J2 (M-1818-100) and MLR2 (M-009-100) either on ice without fixation (top panel), or after fixation with 4% formaldehyde (bottom panel). Both antibodies reveal punctuate membrane staining of p75NTR receptor. This staining is specific, because negative mouse control antibody clone X63 (M-1249-100) does not show immunoreactivity. *Method:* Primary antibodies: 2 µg/mL, 1 hour incubation; Blocking: 10% normal horse serum, 30 minutes; Fixation: 4% formaldehyde, 10 minutes, either before application of primary antibody (bottom panel) or after application of primary antibody (upper panel); Secondary antibody: donkey anti-mouse-CF488A (2 µg/mL), 1 hour incubation. Cell nuclei were stained with Hoechst dye (blue), 1:1,000, 20 minutes at room temperature. Magnification: 100x.