abcam

Product datasheet

Anti-p75 NGF Receptor antibody [EP1039Y] ab52987

Recombinant RabMAb

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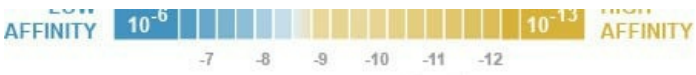
Overview

Product name	Anti-p75 NGF Receptor antibody [EP1039Y]
Description	Rabbit monoclonal [EP1039Y] to p75 NGF Receptor
Host species	Rabbit
Specificity	The mouse and rat recommendation is based on the WB results. We do not guarantee IHC-P for mouse and rat.
Tested applications	**Suitable for:** WB, IP, IHC-P, ICC/IF, Flow Cyt (Intra)
Species reactivity	**Reacts with:** Mouse, Rat, Human
Immunogen	Synthetic peptide within Human p75 NGF Receptor aa 350-450. The exact sequence is proprietary. Database link: **P08138**
Positive control	ICC/IF: PC-12 cells. IHC-P: Human tonsil tissue; Mouse uterus tissue. WB: Capan-1, SW80, Neuro-2a and PC-12 cell lysate; Mouse uterus, hippocampus and cerebral cortex lysate; Rat uterus, hippocampus and brain cortex lysate; Human hippocampus and brain cortex lysate. IP: PC-12 cell lysate. Flow Cyt (intra): PC-12 cells.
General notes	This product is a recombinant monoclonal antibody, which offers several advantages including: - High batch-to-batch consistency and reproducibility - Improved sensitivity and specificity - Long-term security of supply - Animal-free production For more information **see here**. Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to **RabMAb® patents**.

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Upon delivery aliquot and store at -20°C. Avoid freeze / thaw cycles.
Dissociation constant (K_D)	$K_D = 3.25 \times 10^{-10}$ M



Learn more about K_D

Storage buffer	pH: 7.20 Preservative: 0.01% Sodium azide Constituents: PBS, 40% Glycerol (glycerin, glycerine), 0.5% BSA
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EP1039Y
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab52987 in the following tested applications.

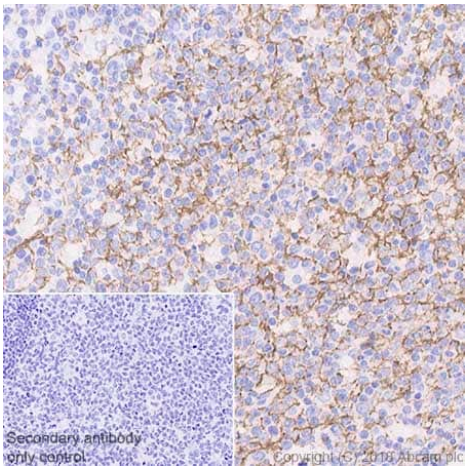
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
WB	★★★★★ (4)	1/1000 - 1/10000. Detects a band of approximately 75 kDa (predicted molecular weight: 45 kDa).
IP		1/50.
IHC-P	★★★★☆ (2)	Use at an assay dependent concentration. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol. The mouse and rat recommendation is based on the WB results. We do not guarantee IHC-P for mouse and rat.
ICC/IF	★★★★★ (1)	1/50.
Flow Cyt (Intra)		1/60.

Target

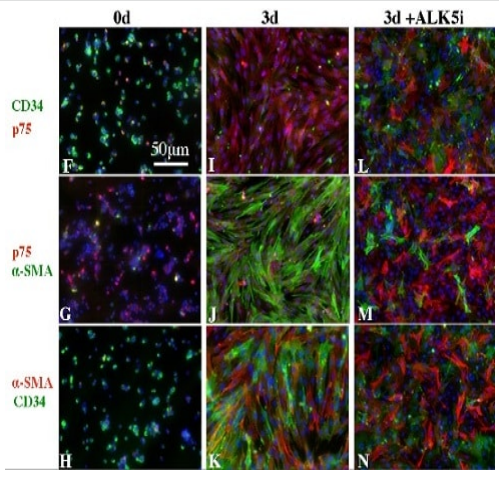
Function	Low affinity receptor which can bind to NGF, BDNF, NT-3, and NT-4. Can mediate cell survival as well as cell death of neural cells.
Sequence similarities	Contains 1 death domain. Contains 4 TNFR-Cys repeats.
Domain	Death domain is responsible for interaction with RANBP9. The extracellular domain is responsible for interaction with NTRK1.
Post-translational modifications	N- and O-glycosylated. O-linked glycans consist of Gal(1-3)GalNAc core elongated by 1 or 2 NeuNAc. Phosphorylated on serine residues.
Cellular localization	Membrane.

Images



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-p75 NGF Receptor antibody [EP1039Y] (ab52987)

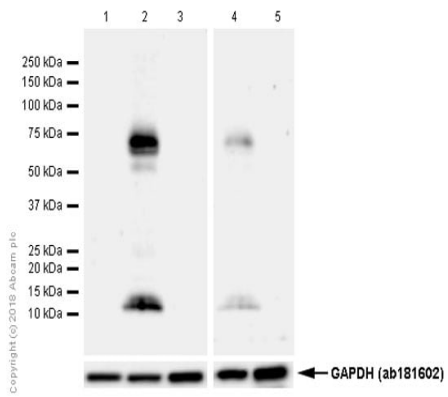
Purified ab52987 staining p75 NGF receptor in paraffin embedded Human tonsil tissue sections by Immunohistochemistry. Antigen retrieval was by heat mediation using **ab93684** (Tris/EDTA buffer, pH 9.0). Samples were incubated with primary antibody at 3.3µg/ml. A ready to use Goat Anti-Rabbit IgG H&L (HRP) was used as the secondary antibody. Hematoxylin was used as a counterstain. Positive staining on germinal centre of human tonsil.



Immunocytochemistry/ Immunofluorescence - Anti-p75 NGF Receptor antibody [EP1039Y] (ab52987)

The differentiation capacity of purified $CD34^+$ cells cultured for 3 days in the presence or absence of ALK5i was evaluated by performing immunofluorescence analysis assessing whether $CD34^+$ cells had changed to cells expressing p75 and/or α-SMA. Expressions of CD34, p75 and α-SMA were assessed by immunofluorescence on day 0 (F-H), day 3 in SP+f medium (I-K), or day 3 in the same medium as (I-K) but with ALK5i (L-N).

Cultured re-aggregates were fixed in 4% PFA and embedded in paraffin. Sections (5 □m) were boiled in 0.01 M citrate (pH 6.0) with 0.1% Tween 20 for 10 min, washed three times in 0.1% Tween-20/PBS, transferred to blocking solution containing 5% BSA and 5% horse serum (Sigma) or goat serum (Invitrogen) in 0.1% Triton X-100/PBS for 1 hr, and incubated with primary antibody (p75 at 1/100 dilution) at 4°C overnight. After washing, the secondary antibody was added, and the sections were incubated for 2 hrs at room temperature. Microscopic images were obtained using a CCD camera (DP72, Olympus, Tokyo) mounted on a fluorescence microscope (BX60, or BX61VS-ASW, Olympus). Cultured cells on coverglasses were fixed in 4% PFA. Antigen retrieval was done by incubation with 100% methanol (-20°C) 10 min, and 0.3% Triton X-100 for 10 min.



Western blot - Anti-p75 NGF Receptor antibody [EP1039Y] (ab52987)

All lanes : Anti-p75 NGF Receptor antibody [EP1039Y] (ab52987) at 1/1000 dilution (purified)

Lane 1 : Capan-1 (Human pancreas adenocarcinoma epithelial cell) whole cell lysates
Lane 2 : Mouse uterus tissue lysates
Lane 3 : Mouse brain tissue lysates
Lane 4 : Rat uterus tissue lysates
Lane 5 : Rat brain tissue lysates

Lysates/proteins at 20 µg per lane.

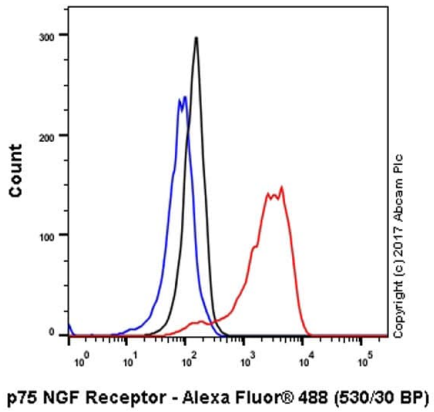
Secondary
All lanes : Goat Anti-Rabbit IgG (HRP) with minimal cross-reactivity with human IgG at 1/2000 dilution

Predicted band size: 45 kDa

Exposure time: 180 seconds

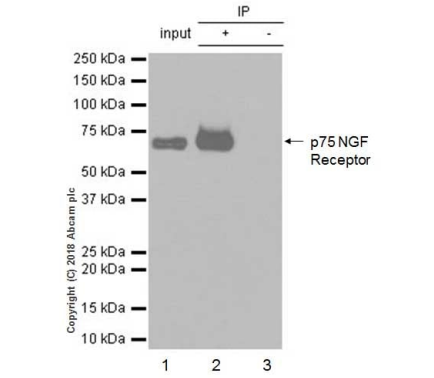
Blocking and diluting buffer: 5% NFDM/TBST

ab52987 fails to detect band of interest in Capan-1 (positive, PMID: 14613990) and brain lysates (positive, PMID: 21413144, 21541365), indicating its low affinity in some p75 NGF Receptor positive materials.



Flow Cytometry (Intracellular) - Anti-p75 NGF Receptor antibody [EP1039Y] (ab52987)

Intracellular Flow Cytometry analysis of PC-12 (rat adrenal gland pheochromocytoma) cells labeling p75 NGF Receptor with purified ab52987 at 1/80 dilution (1ug/mL) (red). Cells were fixed with 4% paraformaldehyde and permeabilised with 90% methanol. A Goat anti rabbit IgG (Alexa Fluor®488) at 1/2000 dilution was used as the secondary antibody. Rabbit monoclonal IgG (**ab172730**) (Black) was used as the isotype control, cells without incubation with primary antibody and secondary antibody (Blue) was used as the unlabeled control.



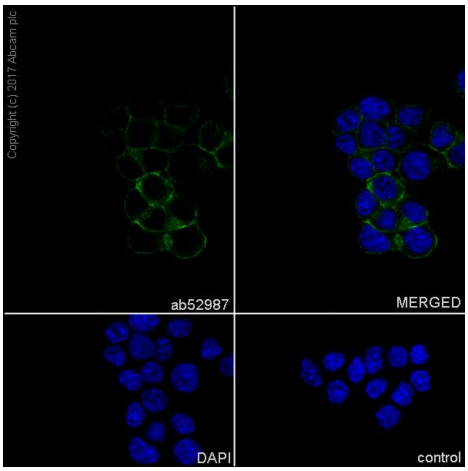
Immunoprecipitation - Anti-p75 NGF Receptor antibody [EP1039Y] (ab52987)

Lane 1 (input): PC-12 (rat adrenal gland pheochromocytoma) whole cell lysate 10µg
Lane 2 (+): PC-12 whole cell lysate
Lane 3 (-): Rabbit monoclonal IgG (**ab172730**) instead of ab52987 in PC-12 whole cell lysate

ab52987 immunoprecipitating p75 NGF receptor in PC-12 whole cell lysates. For western blotting, primary antibody used was ab52987 at 1.6 µg/ml. VeriBlot for IP Detection Reagent (HRP) (**ab131366**), was used for detection at 1/1000 dilution. Capture antibody was used at 1:40 dilution (2µg in 0.35mg lysates).

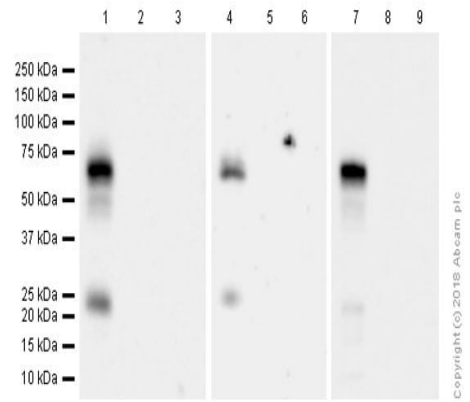
Blocking and diluting buffer: 5% NFDM/TBST.

Exposure: 10 seconds



Immunocytochemistry/ Immunofluorescence - Anti-p75 NGF Receptor antibody [EP1039Y] (ab52987)

Purified ab52987 staining p75 NGF receptor in PC-12 (rat adrenal gland pheochromocytoma) by ICC/IF (Immunocytochemistry/Immunofluorescence). Cells were fixed with 4% paraformaldehyde and permeabilized with 0.1% TritonX-100. Samples were incubated with primary antibody at 3.9 µg/ml. An AlexaFluor®488 Goat anti-Rabbit was used as the secondary antibody at 2 µg/ml. DAPI was used as a nuclear counterstain. Confocal image showing cytoplasmic and Membranous staining in PC-12 cells.



Western blot - Anti-p75 NGF Receptor antibody [EP1039Y] (ab52987)

All lanes : Anti-p75 NGF Receptor antibody [EP1039Y] (ab52987) at 1/1000 dilution (purified)

Lane 1 : SW480 (Human colorectal adenocarcinoma epithelial cell) whole cell lysates
Lane 2 : Human hippocampus tissue lysates
Lane 3 : Human brain cortex tissue lysates
Lane 4 : Neuro-2a (Mouse neuroblastoma neuroblast) whole cell lysates
Lane 5 : Mouse hippocampus tissue lysates
Lane 6 : Mouse cerebral cortex lysates
Lane 7 : PC-12 (Rat adrenal gland pheochromocytoma) whole cell lysates
Lane 8 : Rat hippocampus tissue lysates
Lane 9 : Rat brain cortex tissue lysates

Lysates/proteins at 20 µg per lane.

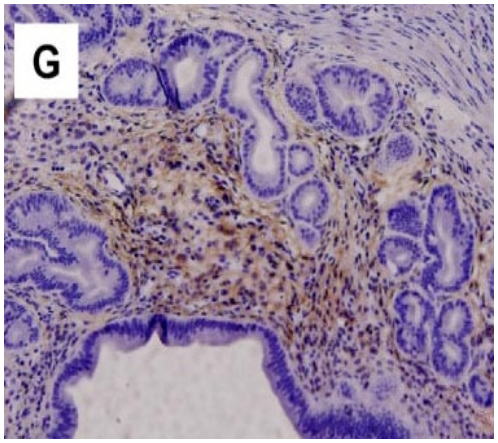
Secondary
All lanes : Goat Anti-Rabbit IgG (HRP) with minimal cross-reactivity with human IgG at 1/2000 dilution

Predicted band size: 45 kDa

Blocking and diluting buffer: 5% NFDM/TBST

ab52987 fails to detect band of interest in hippocampus and cortex lysates (positive, PMID: 25180603, 28507518, 18930453, 20937383, 21059364), indicating its low affinity in some p75 NGF Receptor positive materials.

Exposure: Lane 1-3: 8 seconds
Lane 4-6: 180 seconds
Lane 7-9: 30 seconds

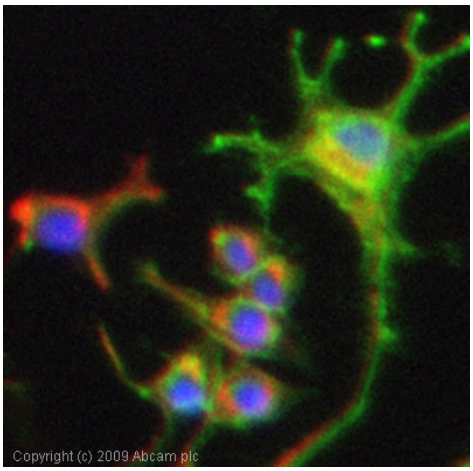


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-p75 NGF Receptor antibody [EP1039Y] (ab52987)

Image from Li Y et al., Reprod Biol Endocrinol. 2011 Mar 8;9:30. Fig 2.; doi:10.1186/1477-7827-9-30; 8 March 2011, Reproductive Biology and Endocrinology 2011, 9:30

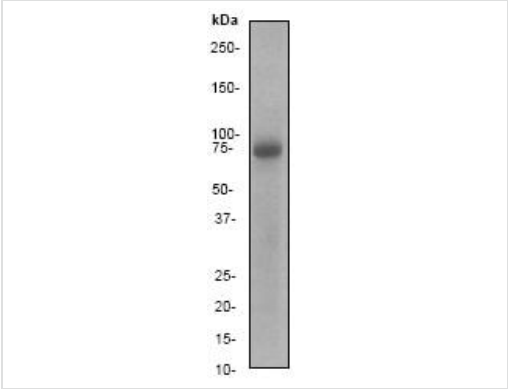
Immunohistochemical analysis of murine uterus tissue with adenomyosis, staining p75 NGF Receptor with ab52987.

Antigen retrieval was performed by heat mediation in citrate buffer (pH 6). Tissue was blocked with goat serum for 15 minutes before incubating with primary antibody (1/100) overnight at 4°C. A biotinylated goat anti-rabbit IgG was used as the secondary antibody and staining was detected using DAB.



Immunocytochemistry/ Immunofluorescence - Anti-p75 NGF Receptor antibody [EP1039Y] (ab52987)

ICC/IF image of ab52987 stained PC12 cells. The cells were 100% methanol fixed (5 min) and then incubated in 1% BSA / 10% normal goat serum / 0.3M glycine in 0.1% PBS-Tween for 1 hour to permeabilise the cells and block non-specific protein-protein interactions. The cells were then incubated with the antibody (ab52987, 1 µg/mL) overnight at 4^{o}C. The secondary antibody (green) was Alexa Fluor® 488 goat anti-rabbit IgG (H+L) used at a 1/1000 dilution for 1 hour. Alexa Fluo® 594 WGA was used to label plasma membranes (red) at a 1/200 dilution for 1 hour. DAPI was used to stain the cell nuclei (blue) at a concentration of 1.4 µM.



Western blot - Anti-p75 NGF Receptor antibody [EP1039Y] (ab52987)

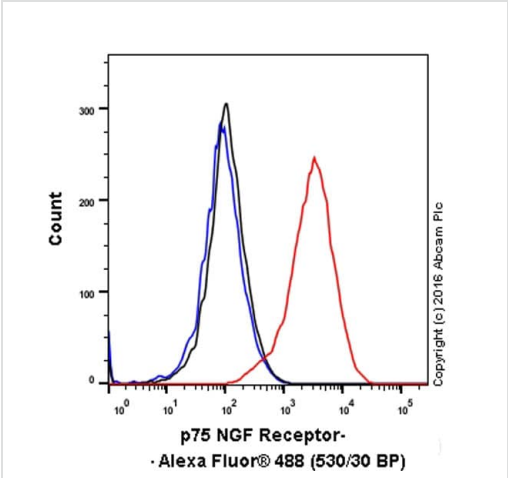
Anti-p75 NGF Receptor antibody [EP1039Y] (ab52987) at 1/50000 dilution + PC12 cell lysate at 10 µg

Secondary

goat anti-rabbit HRP labelled at 1/2000 dilution

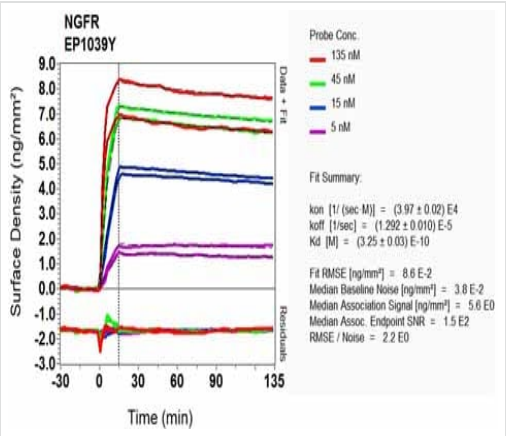
Predicted band size: 45 kDa

Observed band size: 75 kDa



Flow Cytometry (Intracellular) - Anti-p75 NGF Receptor antibody [EP1039Y] (ab52987)

Intracellular Flow Cytometry analysis of PC-12 (rat adrenal gland pheochromocytoma) cells labeling p75 NGF Receptor with unpurified ab52987 at 1/60 dilution (10ug/mL) (red). Cells were fixed with 4% paraformaldehyde and permeabilised with 90% methanol. A Goat anti rabbit IgG (Alexa Fluorr®488) at 1/2000 dilution was used as the secondary antibody. Rabbit monoclonal IgG (Black) was used as the isotype control, cells without incubation with primary antibody and secondary antibody (Blue) was used as the unlabeled control.

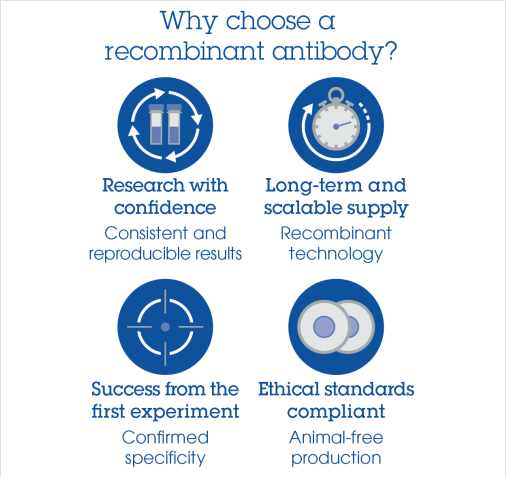


OI-RD Scanning - Anti-p75 NGF Receptor antibody [EP1039Y] (ab52987)

Equilibrium disassociation constant (K_D)

Learn more about K_D

Click here to learn more about K_D



Anti-p75 NGF Receptor antibody [EP1039Y] (ab52987)

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