abcam

Product datasheet

Anti-p75 NGF Receptor antibody [EP1039Y] ab52987

יעלאעבע RabMAb

★★★★★ 11 Abreviews 60 References 画像数 13

製品の概要

製品名 Anti-p75 NGF Receptor antibody [EP1039Y]

製品の詳細 Rabbit monoclonal [EP1039Y] to p75 NGF Receptor

由来種 Rabbit

特異性 The mouse and rat recommendation is based on the WB results. We do not guarantee IHC-P for

mouse and rat.

アプリケーション 適用あり: WB, IP, IHC-P, ICC/IF, Flow Cyt (Intra)

種交差性 交差種: Mouse, Rat, Human

免疫原 Synthetic peptide within Human p75 NGF Receptor aa 350-450. The exact sequence is

proprietary.

Database link: P08138

ポジティブ・コントロール 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.

特記事項 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**.

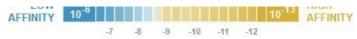
製品の特性

製品の状態 Liquid

保存方法 Shipped at 4°C. Upon delivery aliquot and store at -20°C. Avoid freeze / thaw cycles.

 $K_D = 3.25 \times 10^{-10} M$ 解離定数(KD値)

10-10



Learn more about K_D

バッファー pH: 7.20

Preservative: 0.01% Sodium azide

Constituents: PBS, 40% Glycerol (glycerin, glycerine), 0.5% BSA

精製度 Protein A purified

ポリ/モノ モノクローナル

クローン名 EP1039Y

アイソタイプ lgG

アプリケーション

The Abpromise guarantee <u>Abpromise保証は、</u>次のテスト済みアプリケーションにおけるab52987の使用に適用されます アプリケーションノートには、推奨の開始希釈率がありますが、適切な希釈率につきましてはご検討ください。

アプリケーション	Abreviews	特記事項
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	*** <u>*</u>	1/50.
Flow Cyt (Intra)		1/60.

ターゲット情報

機能 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.

配列類似性 Contains 1 death domain.

Contains 4 TNFR-Cys repeats.

Path domain is responsible for interaction with RANBP9.

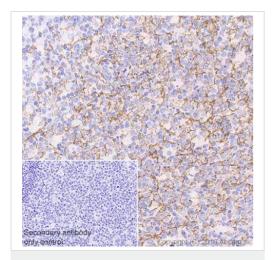
The extracellular domain is responsible for interaction with NTRK1.

翻訳後修飾 N- and O-glycosylated.

O-linked glycans consist of Gal(1-3)GalNAc core elongated by 1 or 2 NeuNAc.

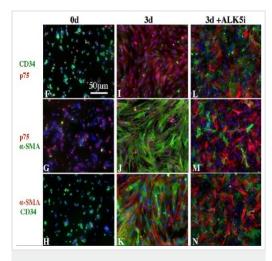
Phosphorylated on serine residues.

細胞内局在 Membrane.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded 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.

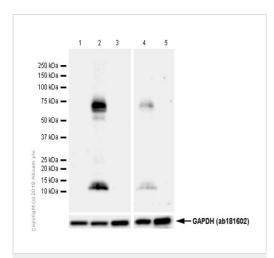


Immunocytochemistry/ Immunofluorescence - Antip75 NGF Receptor antibody [EP1039Y] (ab52987)

Abe, Sl. et al PLoS One. 2017 Nov 30;12(11):e0188705. doi: 10.1371/journal.pone.0188705. eCollection 201 Reproduced under the Creative Commons license http://creativecommons.org/licenses/by/4.0/

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 (FK), or day 3 in the same medium as (FK) 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

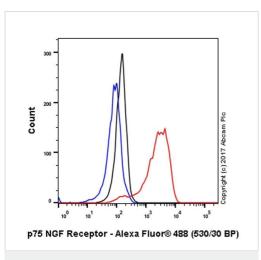
All lanes : Goat Anti-Rabbit $\lg G$ (HRP) with minimal cross-reactivity with human $\lg G$ at 1/2000 dilution

Predicted band size: 45 kDa

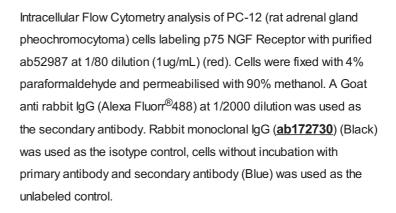
Exposure time: 180 seconds

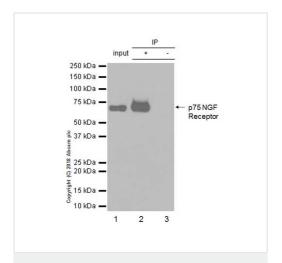
Blocking and diluting buffer: 5% NFDM/TBST

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)





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

Lane 1 (input): PC-12 (rat adrenal gland pheochromocytoma) whole cell lysate 10µg

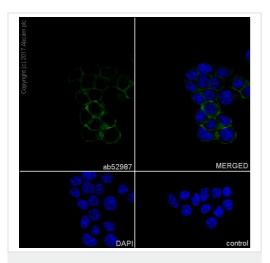
Lane 2 (+): PC-12 whole cell lysate

Lane 3 (-): Rabbit monoclonal IgG (<u>ab172730</u>) 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.

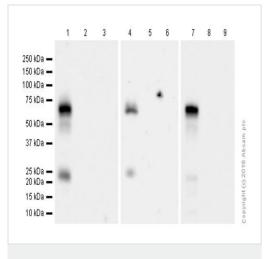
Exposure: 10 seconds



Immunocytochemistry/ Immunofluorescence - Antip75 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 μ g/ml. An AlexaFluor[®]488 Goat anti-Rabbit was used as the secondary antibody at 2 μ g/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

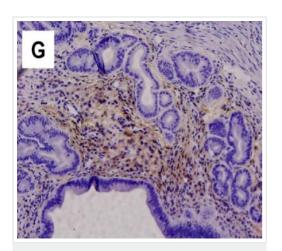
Predicted band size: 45 kDa

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

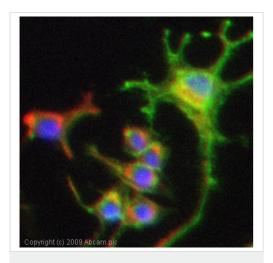
Lane 7-9: 30 seconds



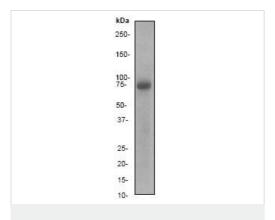
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-p75 NGF Receptor antibody [EP1039Y] (ab52987)

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.

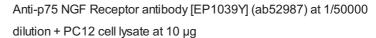
Antigen retrieval was performed by heat mediation in citrate buffer (pH 6). Tissue was blocked with goat serum for 15 minutes before incubating with primary antibody (1/100) overnight at 4°C. A biotinylated goat anti-rabbit IgG was used as the secondary antibody and staining was detected using DAB.



Immunocytochemistry/ Immunofluorescence - Antip75 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 μ g/mL) overnight at 4°C. The secondary antibody (green) was Alexa Fluor® 488 goat anti-rabbit lgG (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 μ M.



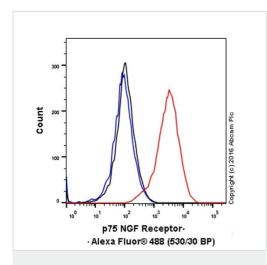
Western blot - Anti-p75 NGF Receptor antibody [EP1039Y] (ab52987)



Secondary

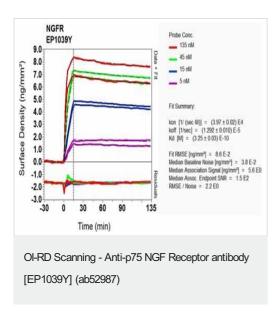
goat anti-rabbit HRP labelled at 1/2000 dilution

Predicted band size: 45 kDa **Observed band size:** 75 kDa



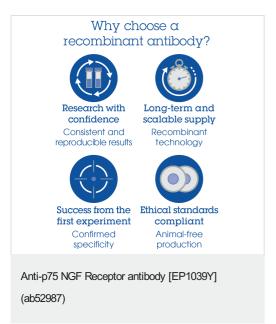
Flow Cytometry (Intracellular) - Anti-p75 NGF Receptor antibody [EP1039Y] (ab52987)

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 lgG (Alexa Fluorr[®]488) at 1/2000 dilution was used as the secondary antibody. Rabbit monoclonal lgG (Black) was used as the isotype control, cells without incubation with primary antibody and secondary antibody (Blue) was used as the unlabeled control.



Equilibrium disassociation constant (K_D) Learn more about K_D

Click here to learn more about K_D



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