abcam

Product datasheet

Anti-Pan Trk antibody [EP1058Y] - BSA and Azide free ab188825



重组 RabMAb

2 References 18 图像

概述

产品名称 Anti-Pan Trk抗体[EP1058Y] - BSA and Azide free

描述 兔单克隆抗体[EP1058Y] to Pan Trk - BSA and Azide free

宿主 Rabbit

特异性 This antibody detects both phosphorylated and unphosphorylated Pan Trk. Based on the WB and

FC data, this antibody has relatively lower affinity to TrkC compared to TrkA and TrkB.

适用于: IP, WB, IHC-P, ICC/IF, Flow Cyt (Intra), Indirect ELISA 经测试应用

种属反应性 与反应: Mouse, Rat, Human

免疫原 Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.

阳性对照 WB: Human, mouse and rat brain tissue lysates IHC-P: Human, mouse and rat cerebrum tissue,

> His-human TrkA/B and C overexpression 293T whole cell pellet. ICC/IF: U87-MG and SH-SY5Y cells; Mouse DRG neurons; mouse primary neuron. Flow Cyt (intra): SH-SY5Y cells. IP: Rat and

mouse brain tissue lysate.

常规说明 ab188825 is the carrier-free version of ab76291.

> Our carrier-free antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. The carrier-free buffer and high concentration allow for increased conjugation efficiency.

This conjugation-ready format is designed for use with fluorochromes, metal isotopes, oligonucleotides, and enzymes, which makes them ideal for antibody labelling, functional and cellbased assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications.

Use our conjugation kits for antibody conjugates that are ready-to-use in as little as 20 minutes with <1 minute hands-on-time and 100% antibody recovery: available for fluorescent dyes, HRP, biotin and gold.

This product is compatible with the Maxpar® Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar[®] is a trademark of Fluidigm Canada Inc.

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**.

性能

形式 Liquid

存放说明 Shipped at 4°C. Store at +4°C. Do Not Freeze.

存储溶液 pH: 7.20

Constituent: PBS

纯**度** Protein A purified

同种型 lgG

应用

The Abpromise guarantee Abpromise™承诺保证使用ab188825于以下的经测试应用

"应用说明"部分下显示的仅为推荐的起始稀释度;实际最佳的稀释度/浓度应由使用者检定。

应用	Ab评论	说明
IP		Use at an assay dependent concentration.
WB		Use at an assay dependent concentration. Predicted molecular weight: 87 kDa.
IHC-P		Use at an assay dependent concentration. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
ICC/IF		Use at an assay dependent concentration.
Flow Cyt (Intra)		Use at an assay dependent concentration. <u>ab199376</u> - Rabbit monoclonal lgG, is suitable for use as an isotype control with this antibody.
Indirect ELISA		Use at an assay dependent concentration.

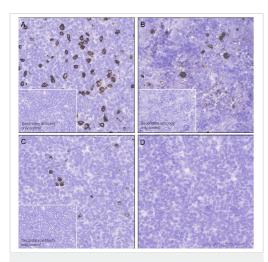
靶标

相关性

Family of neurotrophic tyrosine kinase (NTRK1/2/3) genes which encode TrkA, TrkB and TrkC protein kinases. The three family members are activated by different neurotrophins: TrkA is activated by Nerve growth factor (NGF), TrkB by Brain-derived neurotrophic factor (BDNF) or

neurotrophin-4 (NT-4) and TrkC by NT-3. Neurotrophin signalling activates cellular pathways involved in the development and the maturation of the central and peripheral nervous systems through regulation of proliferation, differentiation and survival of sympathetic and nervous neurons. Localization TrkA: Cell membrane. Early endosome membrane. Late endosome membrane. Internalized to endosomes upon binding of NGF or NT-3 and further transported to the cell body via a retrograde axonal transport. Localized at cell membrane and early endosomes before nerve growth factor (NGF) stimulation. Recruited to late endosomes after NGF stimulation. Colocalized with RAPGEF2 at late endosomes (By similarity). TrkB: Membrane. TrkC: Membrane.

图片



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Pan Trk antibody

[EP1058Y] - BSA and Azide free (ab188825)

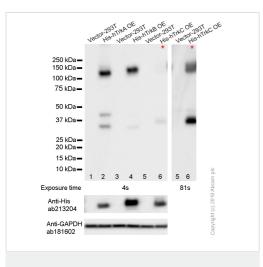
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab76291).

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of (A) His-human TrkA overexpression 293T whole cell pellet, (B) His-human TrkB overexpression 293T whole cell pellet, (C) His-human TrkC overexpression 293T whole cell pellet and (D) HEK-293T transfected with empty plasmid labelling Pan Trk with purified ab76291 at 1/1000. Heat mediated antigen retrieval was performed using Tris-EDTA buffer (pH 9.0, epitope retrieval solution 2) for 20 minutes. Sections were counterstained with hematoxylin.

Positive staining on (A) His-human TrkA overexpression 293T whole cell pellet, (B) His-human TrkB overexpression 293T whole cell pellet and (C) His-human TrkC overexpression 293T whole cell pellet.

No staining on (D) HEK-293T transfected with empty plasmid. The section was incubated with abab76291 for 30 mins at room temperature.

The immunostaining was performed on a Leica Biosystems BOND® RX instrument



Western blot - Anti-Pan Trk antibody [EP1058Y] - BSA and Azide free (ab188825)

All lanes : Anti-Pan Trk antibody [EP1058Y] (ab76291) at 1/1000 dilution

Lanes 1 & 3 & 5 : Empty vector over expression 293T whole cell lysates

Lane 2: His-human TrkA overexpression 293T whole cell lysatesLane 4: His-human TrkB overexpression 293T whole cell lysatesLane 6: His-human TrkC overexpression 293T whole cell lysates

Lysates/proteins at 20 µg per lane.

Secondary

All lanes : Goat Anti-Rabbit IgG H&L (HRP) (<u>ab97051</u>) at 1/20000 dilution

Predicted band size: 87 kDa

Observed band size: 120 kDa

Blocking/Diluting buffer and concentration: 5% NFDM/TBST

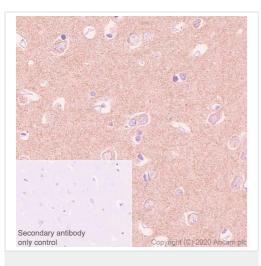
This antibody has relatively lower affinity to TrkC compared to TrkA and TrkB.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab76291).

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human cerebrum tissue labelling Pan Trk with purified ab76291 at 1/1000. Rabbit specific IHC polymer detection kit HRP/DAB (ab209101) was used as the secondary antibody. Heat mediated antigen retrieval was performed using Tris-EDTA buffer (pH 9.0, epitope retrieval solution 2) for 20 minutes. Sections were counterstained with hematoxylin. Negative control using PBS instead of primary antibody.

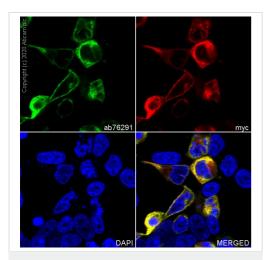
Positive staining on human cerebrum.

The section was incubated with <u>ab76291</u> for 30 mins at room temperature. The immunostaining staining was performed on a Leica Biosystems BOND[®] RX instrument.

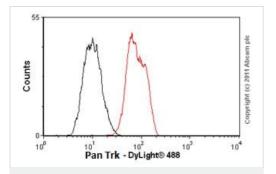


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Pan Trk antibody

[EP1058Y] - BSA and Azide free (ab188825)



Immunocytochemistry/ Immunofluorescence - Anti-Pan Trk antibody [EP1058Y] - BSA and Azide free (ab188825)



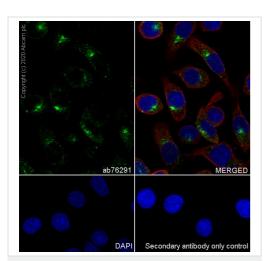
Flow Cytometry (Intracellular) - Anti-Pan Trk antibody [EP1058Y] - BSA and Azide free (ab188825)

Immunocytochemsitry/Immunofluorescence analysis of 293T (human embryonic kidney epithelial cell) cells labelling Pan Trk with ab76291 at 1/1000 dilution (0.7 μg/mL). ab150077, AlexaFluor[®]488 Goat anti-Rabbit secondary at 1/1000 (2 μg/mL) was used as the secondary antibody. Cells were fixed with 4% Paraformaldehyde and permeabilised with 0.1% TritonX-100. DAPI (blue) was used as nuclear counterstain. Cells were counterstained with Myc-Tag (9B11) Mouse mAb (Alexa Fluor[®] 647 Conjugate) at 1/200 (2.5 μg/mL).

Confocal image showing cytoplasmic staining in 293T cells transfected with a myc-tagged hTrkA expression vector.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab76291).

Overlay histogram showing SH-SY5Y cells stained with unpurified ab76291 (red line). The cells were fixed with 4% paraformaldehyde (10 min) and incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions. The cells were then incubated with the antibody (ab76291, 1/50 dilution) for 30 min at 22°C. The secondary antibody used was DyLight[®] 488 goat anti-rabbit IgG (H+L) (ab96899) at 1/500 dilution for 30 min at 22°C. Isotype control antibody (black line) was rabbit monoclonal IgG (1µg/1x10⁶ cells) used under the same conditions. Acquisition of >5,000 events was performed. This antibody gave a positive signal in SH-SY5Y cells fixed with methanol (5 min) used under the same conditions. Please note that Abcam do not have data for use of this antibody on non-fixed cells. We welcome any customer feedback.

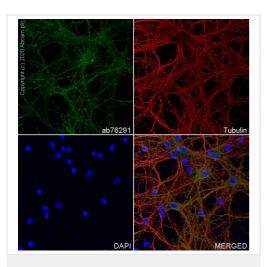


Immunocytochemistry/ Immunofluorescence - Anti-Pan Trk antibody [EP1058Y] - BSA and Azide free (ab188825)

Immunocytochemistry/immunofluorescence analysis of SH-SY5Y (Human neuroblastoma epithelial cell) labeling pan Trk with $\underline{ab76291}$ at 1/100 dilution (7 µg/mL). $\underline{ab150077}$, AlexaFluor $^{\$}488$ Goat anti-Rabbit secondary at 1/1000 (2 µg/mL) was used as the secondary antibody. Cells were fixed with 4% Paraformaldehyde and permeabilised with 0.1% TritonX-100. DAPI (blue) was used as nuclear counterstain. Cells were counterstained with $\underline{ab195889}$, anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor $^{\$}594$) at 1/200 (2.5 µg/mL).

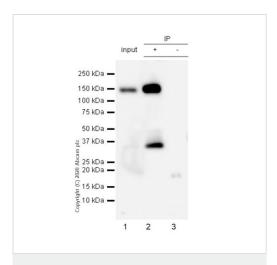
Confocal image showing cytoplasmic staining in SH-SY5Y cell line.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab76291).



Immunocytochemistry/ Immunofluorescence - Anti-Pan Trk antibody [EP1058Y] - BSA and Azide free (ab188825)

Immunofluorescent analysis of 4% Paraformaldehyde-fixed, 0.1% TritonX-100 permeabilized mouse primary neuron cells labelling Pan Trk with ab76291 at 1/100 dilution, followed by ab150077 AlexaFluor[®] 488 Goat anti-Rabbit secondary antibody at 1/1000 dilution (Green). Confocal scanning Z step was set as 0.3 µm followed by image processing with maximum Z projection.
ab195889 Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor[®] 594) was used to counterstain tubulin at 1/200 dilution (Red). The Nuclear counterstain was DAPI (Blue).



Immunoprecipitation - Anti-Pan Trk antibody

[EP1058Y] - BSA and Azide free (ab188825)

<u>ab76291</u> at 1/40 immunoprecipitating Pan Trk in mouse brain tissue lysate observed at 145 kDa.

Lane 1 (input): Mouse brain tissue lysate (10µg)

Lane 2 (+): ab76291+ mouse brain tissue lysate.

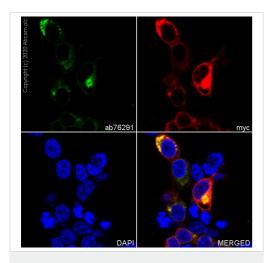
Lane 3 (-): Rabbit monoclonal $\lg G$ (ab172730) instead of ab76291 in Mouse brain lysate

For western blotting, <u>ab76291</u> at 1/1000 dilution (0.7 μ g/mL) and VeriBlot for IP Detection Reagent (HRP) (<u>ab131366</u>) at 1/5000 were used.

The 30 kDa band is an intracellular fragment, and the 140 kDa observed MW which is higher than the predicted one is due to the glycosylation modification. (refer to ab189903).

Blocking/Diluting buffer and concentration: 5% NFDM/TBST.

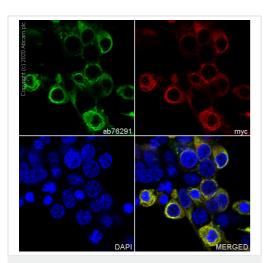
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab76291).



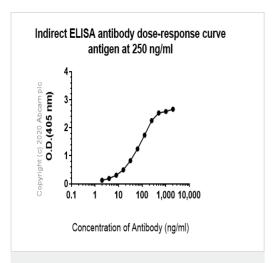
Immunocytochemistry/ Immunofluorescence - Anti-Pan Trk antibody [EP1058Y] - BSA and Azide free (ab188825)

Immunocytochemsitry/Immunofluorescence analysis of 293T (human embryonic kidney epithelial cell) cells labelling Pan Trk with ${\tt ab76291}$ at 1/500 dilution (1.4 ${\tt \mug/mL}$). ${\tt ab150077}$, AlexaFluor [®] 488 Goat anti-Rabbit secondary at 1/1000 (2 ${\tt \mug/mL}$) was used as the secondary antibody. Cells were fixed with 4% Paraformaldehyde and permeabilised with 0.1% TritonX-100. DAPI (blue) was used as nuclear counterstain. Cells were counterstained with Myc-Tag (9B11) Mouse mAb (Alexa Fluor [®] 647 Conjugate) at 1/200 (2.5 ${\tt \mug/mL}$).

Confocal image showing cytoplasmic staining in 293T cells transfected with a myc-tagged hTrkC expression vector.



Immunocytochemistry/ Immunofluorescence - Anti-Pan Trk antibody [EP1058Y] - BSA and Azide free (ab188825)



Indirect ELISA - Anti-Pan Trk antibody [EP1058Y] - BSA and Azide free (ab188825)

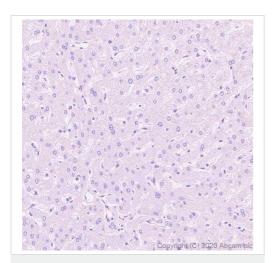
AlexaFluor[®]488 Goat anti-Rabbit secondary at 1/1000 (2 μg/mL) was used as the secondary antibody. Cells were fixed with 4% Paraformaldehyde and permeabilised with 0.1% TritonX-100. DAPI (blue) was used as nuclear counterstain. Cells were counterstained with Myc-Tag (9B11) Mouse mAb (Alexa Fluor[®] 647 Conjugate) at 1/200 (2.5 μg/mL).

Confocal image showing cytoplasmic staining in 293T cells transfected with a myc-tagged hTrkB expression vector.

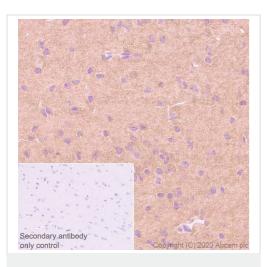
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab76291).

This data was developed using <u>ab76291</u>, the same antibody clone in a different buffer formulation.

ELISA analysis of Human TrkA recombinant protein at 250 ng/mL with <u>ab76291</u>. An Alkaline Phosphatase-conjugated AffiniPure Goat Anti-Rabbit lgG (H+L) at 1/2500 dilution was used as the secondary antibody.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Pan Trk antibody [EP1058Y] - BSA and Azide free (ab188825)



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Pan Trk antibody

[EP1058Y] - BSA and Azide free (ab188825)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human liver tissue labelling Pan Trk with purified ab76291 at 1/1000. Rabbit specific IHC polymer detection kit HRP/DAB (ab209101) was used as the secondary antibody. Heat mediated antigen retrieval was performed using Tris-EDTA buffer (pH 9.0, epitope retrieval solution 2) for 20 minutes. Sections were counterstained with hematoxylin. Negative control using PBS instead of primary antibody.

Negative control: No staining on human liver.

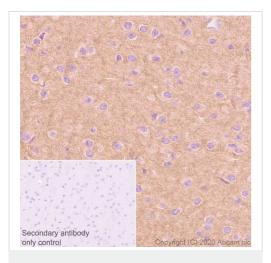
The section was incubated with <u>ab76291</u> for 30 mins at room temperature. The immunostaining staining was performed on a Leica Biosystems BOND[®] RX instrument.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (<u>ab76291</u>).

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of rat cerebrum tissue labelling Pan Trk with purified ab76291 at 1/1000. Rabbit specific IHC polymer detection kit HRP/DAB (ab209101) was used as the secondary antibody. Heat mediated antigen retrieval was performed using Tris-EDTA buffer (pH 9.0, epitope retrieval solution 2) for 20 minutes. Sections were counterstained with hematoxylin. Negative control using PBS instead of primary antibody.

Positive staining on rat cerebrum.

The section was incubated with <u>ab76291</u> for 30 mins at room temperature. The immunostaining staining was performed on a Leica Biosystems BOND[®] RX instrument.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Pan Trk antibody

[EP1058Y] - BSA and Azide free (ab188825)

ab76291 MERGED

Immunocytochemistry/ Immunofluorescence - Anti-Pan Trk antibody [EP1058Y] - BSA and Azide free (ab188825)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of mouse cerebrum tissue labelling Pan Trk with purified ab76291 at 1/1000. Rabbit specific IHC polymer detection kit HRP/DAB (ab209101) was used as the secondary antibody. Heat mediated antigen retrieval was performed using Tris-EDTA buffer (pH 9.0, epitope retrieval solution 2) for 20 minutes. Sections were counterstained with hematoxylin. Negative control using PBS instead of primary antibody.

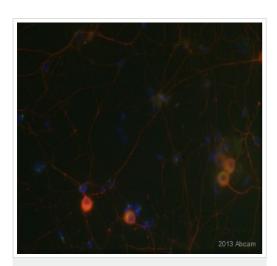
Positive staining on mouse cerebrum.

The section was incubated with <u>ab76291</u> for 30 mins at room temperature. The immunostaining staining was performed on a Leica Biosystems BOND[®] RX instrument.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab76291).

Immunocytochemistry/Immunofluorescence analysis of U87-MG cells labelling Pan Trk with purified <u>ab76291</u> at 1/100. Cells were fixed with 4% paraformaldehyde and permeabilized with 0.1% Triton X-100. An Alexa Fluor[®] 555-conjugated goat anti-rabbit IgG (1/500) was used as the secondary antibody. DAPI (blue) was used as the nuclear counterstain.

Control: primary antibody (1/100) and secondary antibody, **ab150113**, an Alexa Fluor[®] 488-conjugated goat anti-mouse IgG (1/500).



Immunocytochemistry/ Immunofluorescence - Anti-Pan Trk antibody [EP1058Y] - BSA and Azide free (ab188825)

This image is courtesy of an Abreview submitted by Franziska Denk.

ICC/IF image of Pan Trk staining on culture of mouse DRG neurons using unpurified ab76291 (1/100). The cells were fixed using formaldehyde and permeabilized using 0.2% Triton X-100. The cells were blocked using 10% Goat serum for 1 hour at 22°C. Unpurified ab76291 was diluted 1/100 using PBS and incubated with the cells for 30 mins at 22°C. The secondary antibody used was Goat polyclonal to Rabbit IgG conjugated to Alexa Fluor[®] 488 (1/1000). Neuron was stained using Beta III tubulin antibody (Alexa Fluor[®] 647)

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab76291).



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Pan Trk antibody

[EP1058Y] - BSA and Azide free (ab188825)

This image is courtesy of an anonymous Abreview.

Unpurified <u>ab76291</u> staining Pan Trk in murine brain tissue by Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections).

Tissue was fixed with formaldehyde, permeabilized with 0.1% Saponin/PBS and blocked with 4% serum for 30 minutes at 25°C, antigen retrieval was by heat mediation with a citrate buffer. Samples were incubated with primary antibody (1/150 in blocking buffer) for 16 hours at 4°C. A FITC-conjugated goat anti-rabbit polyclonal IgG (1/100) was used as the secondary antibody.



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