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Anti-Phospho-TrkB (Y817) NTRK2 Rabbit Monoclonal Antibody

Catalog Number: P01388

Overview

Product Name	Anti-Phospho-TrkB (Y817) NTRK2 Rabbit Monoclonal Antibody
Reactive Species	Human, Mouse, Rat
Description	Boster Bio Anti-Phospho-TrkB (Y817) NTRK2 Rabbit Monoclonal Antibody catalog # P01388. Tested in WB, IHC, ICC/IF, IP applications. This antibody reacts with Human, Mouse, Rat.
Application	IP, IF, IHC, ICC, WB
Clonality	Monoclonal EGA-14
Formulation	Rabbit IgG in stabilizing components, phosphate buffered saline, pH 7.4, 150mM NaCl, 0.02% sodium azide and 50% glycerol. *This antibody is supplied in a stabilized formulation. Compatibility with conjugation reactions depends on the chemistry of the conjugation method used. For conjugation methods that are not compatible with the stabilizing components present in this formulation, a carrier-free antibody format is required.
Storage Instructions	Store at -20°C for one year. For short term storage and frequent use, store at 4°C for up to one month. Avoid repeated freeze-thaw cycles.
Host	Rabbit
Uniprot ID	Q16620

Technical Details

Immunogen	A synthesized peptide derived from human Phospho-TrkB (Y817)
Isotype	Rabbit IgG
Form	Liquid
Concentration	0.5mg/ml
Purification	Affinity-chromatography
Suggested Dilutions	WB 1:500-2000 IHC 1:50-200 ICC/IF 1:50-200 IP 1:30

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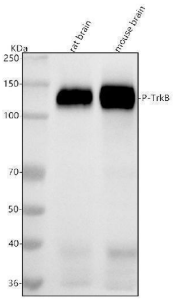
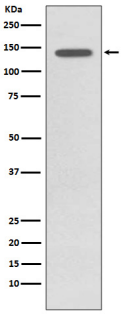
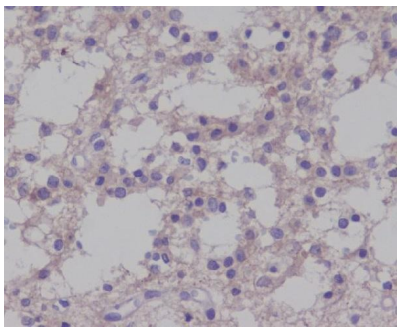
Anti-Phospho-TrkB (Y817) NTRK2 Rabbit Monoclonal Antibody (P01388) Images



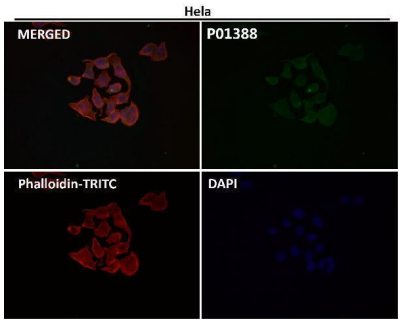
Western blot analysis of TrkB using anti-TrkB antibody (P01388). Electrophoresis was performed on a 5-20% SDS-PAGE gel at 70V (Stacking gel) / 90V (Resolving gel) for 2-3 hours. The sample well of each lane was loaded with 30 ug of sample under reducing conditions. Lane 1: rat brain tissue lysates, Lane 2: mouse brain tissue lysates. After electrophoresis, proteins were transferred to a nitrocellulose membrane at 150 mA for 50-90 minutes. Blocked the membrane with 5% non-fat milk/TBS for 1.5 hour at RT. The membrane was incubated with rabbit anti-TrkB antigen affinity purified monoclonal antibody (Catalog # P01388) at 1:500 overnight at 4°C, then washed with TBS-0.1%Tween 3 times with 5 minutes each and probed with a goat anti-rabbit IgG-HRP secondary antibody at a dilution of 1:500 for 1.5 hour at RT. The signal is developed using an Enhanced Chemiluminescent detection (ECL) kit (Catalog # EK1002) with Tanon 5200 system. A specific band was detected for TrkB at approximately 130 kDa. The expected band size for TrkB is at 92 kDa.



Western blot analysis of Phospho-TrkB (Y817) expression in SH-SY5Y cell lysate treated with BDNF.



Immunohistochemical analysis of paraffin-embedded mouse brain cancer, using Phospho-TrkB (Y817) Antibody.



Immunofluorescent analysis using the Antibody at 1:50 dilution.

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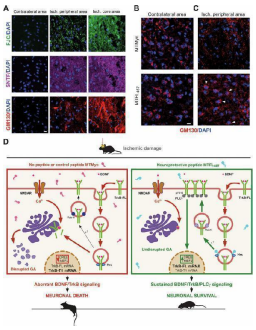

Effect of brain ischemia on GA stability, MTFL 457 regulation, and proposed model. A Strong association between neuronal degeneration and serum protein leakage after ischemic damage. Immunohistochemistry of brain coronal sections from animals sacrificed 5 h after insult was performed with an antibody recognizing a calpain-generated neoepitope in spectrin N-terminal fragment (SNTF, magenta), labeling cells where this protease is overactive, and a mouse antibody recognizing GM130 (red). Neurodegeneration was also detected by Fluoro-Jade C (FJC) staining (green). Three different tissue areas were compared: the ischemic core, an area peripheral to the infarct core, and the equivalent area of the contralateral hemisphere. Leakage of mouse immunoglobulins due to early blood-brain barrier (BBB) breakage after the ischemic insult, detected by the secondary anti-mouse antibody (see Fig. S), was observed in the neurodegenerating tissue and strongly interfered with GM130 detection. B , C GM130 staining after preincubation of coronal sections with an anti-mouse IgG (Fab specific) antibody to improve detection. Animals were retro-orbitally injected with peptides MTMyc or MTFL 457 (10 nmol/g) 10 min after damage initiation and sacrificed 5 h later. Comparison of the contralateral (B) and the ischemic peripheral areas (C). Representative images correspond to single sections. Scale bar: 10 µm. D Model of TrkB-FL regulation in excitotoxicity and MTFL 457 action. Endocytosis of neurotrophin receptor TrkB-FL is promoted by excitotoxicity in neurons treated with control peptide MTMyc or without treatment (left panel). In endosomes, TrkB-FL interacts with the protein Hrs and is retrogradely transported to the Golgi apparatus (GA), where activation of organelle-associated proteinases would be responsible for receptor processing by calpain and regulated intramembrane proteolysis (RIP). Although partial recycling back to the membrane might occur via mechanisms similar to those found after BDNF activation, there is a strong decrease in BDNF/TrkB-FL signaling and CREB/MEF2 promoter activities, causing transcriptional changes that favor neuronal death. In parallel, the GA is disrupted, a hallmark common to many neurodegenerative diseases (NDDs). The neuroprotective peptide MTFL 457 interferes with the TrkB-FL/Hrs interaction induced by excitotoxicity, receptor retrograde transport and processing, as well as GA fragmentation (right panel). We propose that interference by MTFL 457 with the TrkB-FL/Hrs interaction might favor rapid recycling back to the membrane, similar to that of isoform TrkB-T1, sustained BDNF/TrkB-FL/PLCgamma signaling, and promotion of neuronal survival. Index in PubMed under a CC BY license. PMID: 40883288

Effect of excitotoxicity on TrkB-FL transport to the Golgi complex and regulation by MTFL 457 action. Cortical neurons were treated with NMDA for the indicated times and analyzed by immunofluorescence with antibodies specific for the Golgi matrix protein GM130 (green) and TrkB-FL Ct (red); nuclear staining was performed with DAPI (blue). Excitotoxicity was induced in the absence of peptides (A – C) or after preincubation with MTMyc and MTFL 457 (25 uM,

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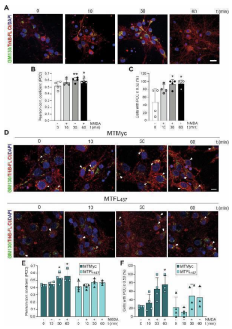
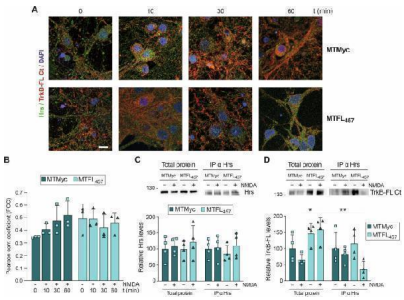
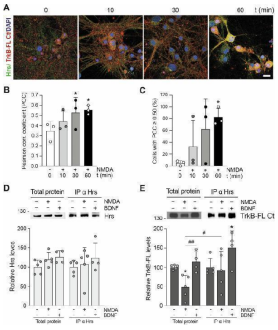


30 min) (D – F). A , D Representative images obtained by confocal microscopy corresponding to single sections, showing channels fused. Scale bar: 20 um. B , E Mean PCC values ± SD (0-30 min, n = 4; 60 min, n = 3) for TrkB-FL and GM130 colocalization. Statistical analysis was performed using a generalized linear model followed by a post-hoc Fisher's LSD test (* P



Regulation by MTFL 457 of the TrkB-FL/Hrs interaction induced by excitotoxicity. A Cortical neurons were preincubated with MTMyc and MTFL 457 (25 uM, 30 min) and treated with NMDA for the indicated times. Cells were analyzed by immunofluorescence with antibodies for Hrs (green) and TrkB-FL Ct (red), together with DAPI staining (blue). Representative confocal microscopy images correspond to single sections and show the fused channels. Scale bar: 10 um. B Mean values ± SD of Pearson correlation coefficient (PCC; n = 3). For each independent experiment, a minimum of 80 different neurons were analyzed. Statistical analysis was performed using a generalized linear model followed by a post-hoc Fisher's LSD test (P = 0.06, 0 vs. 60 min of NMDA treatment in MTMyc-cultures). C , D Analysis by immunoprecipitation of TrkB-FL/Hrs interaction. Cultures preincubated with cell-penetrating peptides (CPPs) as above were treated with NMDA for 30 min and compared to untreated cultures. Immunoprecipitation (IP) was performed with the Hrs antibody, and the immunoprecipitated proteins were analyzed by immunoblot using the same antibody (C) or TrkB-FL Ct (D). Total protein lysates and immunoprecipitated proteins were analyzed in parallel. Mean values ± SD (n = 4) of Hrs and TrkB-FL levels relative to those found in cells preincubated with MTMyc and without NMDA are represented. Statistical analysis was performed using two-way ANOVA followed by a Bonferroni test (* P



Effect of excitotoxicity on TrkB-FL interaction with endosomal protein Hrs. A – C Analysis by immunofluorescence of TrkB-FL/Hrs colocalization. A Cortical neurons were treated with NMDA for the indicated times and analyzed with antibodies specific for Hrs (green) and TrkB-FL Ct (red); nuclear staining was performed with DAPI (blue). Representative images obtained by confocal microscopy correspond to single sections and show the fused channels. Scale bar: 20 um. B Mean values ± SD of Pearson correlation coefficient (PCC; n = 3). For each independent experiment, a minimum of 80 different neurons were analyzed. Statistical analysis was performed using a generalized linear model followed by a post-hoc Fisher's LSD test (* P

MTFL 457 preserves pY816-TrkB-FL at the cell surface, protecting it from proteolytic machinery activated secondarily by excitotoxicity. A – D Kinetics of TrkB-FL downregulation. Primary cortical cultures were treated with

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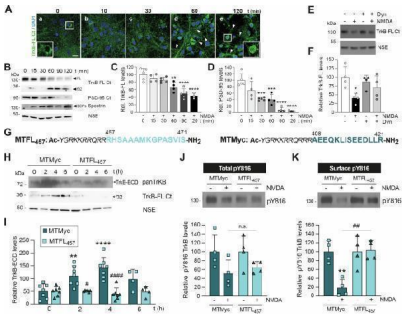
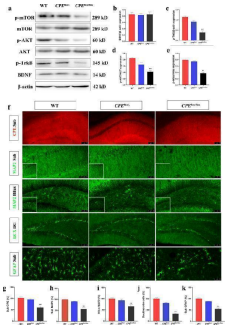


100 uM NMDA and its co-agonist 10 uM glycine (hereafter referred to as 'NMDA'). Immunofluorescence (A) and immunoblotting (B) used a C-terminal (C-ter) isoform-specific antibody (TrkB-FL Ct) recognizing both the full-length protein (FL) and the intracellular fragment (f32). A Shows TrkB-FL (green) and nuclei (blue, DAPI stain). Arrowheads indicate varicosities in neuronal projections. Scale bar: 20 um. Insets show cell body details for untreated cells and cells treated with NMDA for 120 min. B Compares the decrease in TrkB-FL and formation of f32 with PSD-95 downregulation, detected using a C-terminal antibody (PSD-95 Ct). Calpain activation was confirmed by the accumulation of characteristic spectrin breakdown products (BDPs; 150 and 145 kDa). Neuron-specific enolase (NSE) served as a loading control for protein normalization. C , D Quantification of normalized TrkB-FL and PSD-95 levels, shown relative to levels in the absence of NMDA (control). Data are represented as means ± SD. Statistical analysis: one-way analysis of variance (ANOVA) followed by Bonferroni post hoc test (** P



Decreased hippocampal dentate gyrus (DG) neurogenesis in CPE flox/flox mice. a – e Western blot analysis of p -TrkB, BDNF, p -mTOR, mTOR, p -AKT, and AKT levels in the hippocampus. f Immunofluorescence of CPE, MAP2, DCX, and GFAP; and the relative fluorescent intensities of g CPE in Sub, h MAP2 in Sub, i MAP2 in hilius, j DCX in DG, k GFAP in DG of WT, CPE flox/− , and CPE flox/flox mice at 100× and 400× (square in the panel). n = 6; * P < 0.05 and ** P < 0.01 compared with WT; values are mean ± SEM. Index in PubMed under a CC BY license. PMID: 37100779

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