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PRODUCT DATASHEET

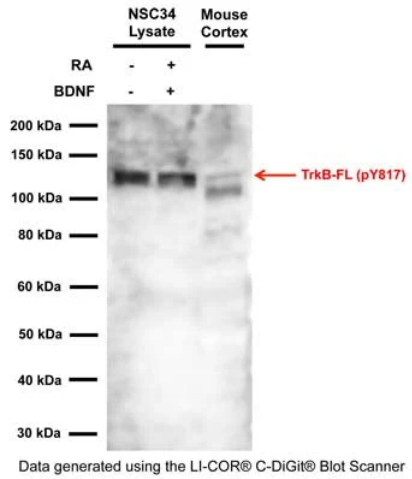
Anti-TrkB phospho Tyr816/Y817 Antibody

Product Details

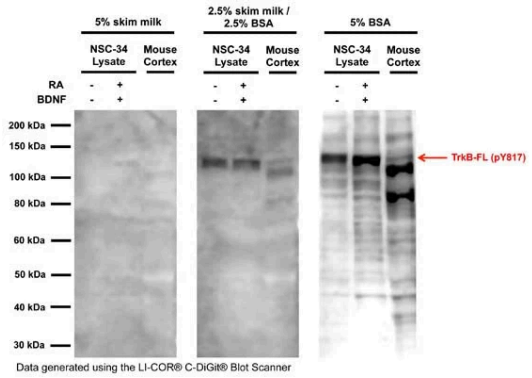
Available Variants	50 µg (SKU:R-1717-50)
Conjugate	Unconjugated
Isotype	IgG
Host Species	Rabbit
Format	Affinity-purified
Physical State	Lyophilized
Buffer	PBS, pH 7.2-7.6, 0.1% trehalose, without preservatives.
Production Notes	Affinity purified, and absorbed.
Applications	WB
Dilution Ranges	WB: 0.5-2 µg/mL
Species Reactivity	Chicken, Human, Mouse, Rat
Immunogen	Synthetic peptide immunogen, AKASPV[pY]LDILG
Specificity	Antibody detects a clear band in retinoic acid (RA) and BDNF-treated NSC34 cell lysates at ~120 kDa only. Human TrkB (pY817). Antibody has been shown to be specific for TrkB phosphorylated on tyrosine 817 by phospho-peptide absorption dot blots, and on cell lysates from cell lines induced with retinoic acid and BDNF. Antibody detects a clear band in retinoic acid (RA) and BDNF-treated NSC34 cell lysates at ~120 kDa only, demonstrating that the phosphorylated TrkB receptor is being detected. While not fully tested, this antibody may detect phosphorylated TrkA (pY791/794, human/rodent) and TrkC (pY834/820/859, human/mouse/rat) due to high degree of amino acid homology surrounding the phosphorylation site.
Molecular Weight	120 kDa

Storage	Spin vial briefly before opening. Reconstitute in 50 µL sterile-filtered, ultrapure water. 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	Q16620
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



Western blot analysis of TrkB pY817 expression in NSC34 RIPA cell lysate (10 µg/lane) and mouse cortex homogenate (30 µg/lane). NSC34 cells were grown in proliferation medium in presence and absence of Retinoic Acid (RA, 1 µM) for 5 days. Cells were then serum-starved for 4 hours and treated with 50 ng/mL BDNF for 30 min. SDS-PAGE: 4-12%, reducing conditions; Transfer: Towbin's Tris-Glycine buffer, PVDF membrane (0.45 µm); Blocking Buffer and antibody diluent: 2.5% skim milk/2.5% BSA in TBST, blocking 1 hour at RT; Primary antibody: 1 µg/mL, overnight at 2-8°C; Secondary antibody: anti-rabbit-HRP (1/10,000) 2.5 hours at RT; Detection: Chemiluminiscence.



Optimization of blocking buffer for **Western blot** analysis of TrkB pY817 expression in NSC34 RIPA cell lysate (10 µg/lane) and mouse cortex homogenate (30 µg/lane), using rabbit antibody R-1717-50. NSC34 cells were grown in proliferation medium in presence and absence of Retinoic Acid (RA, 1 µM) for 5 days. Cells were then serum-starved for 4 hours and treated with 50 ng/mL BDNF for 30 min. SDS-PAGE: 4-12%, reducing conditions; Transfer: Towbin's Tris-Glycine buffer, PVDF membrane (0.45 µm); Blocking Buffer and antibody diluent: as shown in image, blocking 1 hour at RT; Primary antibody: 1 µg/mL, overnight at 2-8°C; Secondary antibody: anti-rabbit-HRP (1/10,000) 2.5 hours at RT; Detection: Chemiluminiscence.

High skim milk concentration causes suppression of pY817 signal, suggesting that 5% skim milk should be avoided as blocking buffer and diluent. Strongest signal is obtained in 5% BSA blocking buffer, however, many non-specific bands are present. An equal mixture of skim milk and BSA appears to provide the best compromise between signal and noise. However, optimization of blocking condition is recommended for each particular sample, with excess of BSA over skim milk likely to be beneficial for best results.