
Certificate of Analysis

Anti-TrkB

(rabbit polyclonal IgG)

Catalog # 07-225

Lot #JBC1361713

Immunogen: The entire extracellular domain (corresponding to residues 1–429) of the rat TrkB receptor, expressed in COS cells. The immunizing sequence has 97% identity with mouse TrkB and 88% identity with human TrkB.

Specificity: Recognizes TrkB, Mr 145 kDa, as well as an additional protein, Mr 90 kDa, which is likely a TrkB degradation product.

Species Cross-reactivity: Rat and mouse.

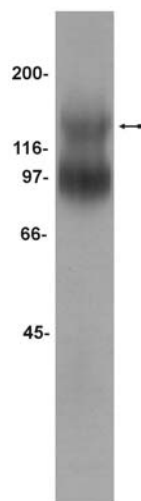
Formulation: 200 µg of protein A purified rabbit IgG in 200 µL 0.014M phosphate buffer, pH 7.6, 0.175M NaCl, 0.07% sodium azide, and 30% glycerol. Liquid at -20°C.

Storage and Stability: Stable for 2 years at -20°C from date of shipment. For maximum recovery of product, centrifuge the vial prior to removing the cap.

**FOR RESEARCH USE ONLY
NOT FOR USE IN HUMANS**

Quality Control Testing

Immunoblot Analysis: 0.5-2 µg/mL of this lot detected TrkB in mouse brain membrane protein preparations.



Immunoblot Analysis

Representative blot from a previous lot. Mouse brain membrane protein preparation was resolved by electrophoresis, transferred to nitrocellulose, and probed with anti-TrkB (1 µg/mL). Proteins were visualized using a goat anti-rabbit secondary antibody conjugated to HRP and a chemiluminescence detection system. Arrow indicates TrkB (~145 kDa).

General References:

1. Huang, E J, *et al* (1999). Expression of Trk receptors in the developing mouse trigeminal ganglion: in vivo evidence for NT-3 activation of TrkA and TrkB in addition to TrkC. *Development* **126**: 2191-203.
2. Clary, D O, *et al* (1994). TrkA cross-linking mimics neuronal responses to nerve growth factor. *Mol Biol Cell* **5**: 549-63.
3. Weskamp, G and Reichardt, L F (1991). Evidence that biological activity of NGF is mediated through a novel subclass of high affinity receptors. *Neuron* **6**: 649-63.

Immunoblot Protocol

1. Perform SDS-polyacrylamide gel electrophoresis (SDS-PAGE) on a cell lysate sample (cell lysis buffer: 50 mM Tris-HCl, pH 7.4; 1% NP-40; 0.25% sodium deoxycholate; 150 mM NaCl; 1 mM EGTA; 1 mM PMSF; 1 μ g/mL each aprotinin, leupeptin, pepstatin; 1 mM Na_3VO_4 ; 1 mM NaF) and transfer the proteins to nitrocellulose. Wash the blotted nitrocellulose twice with water.
2. Incubate the nitrocellulose with 0.1% Tween in TBS for 10 minutes at room temperature with constant agitation.
3. Block the blotted nitrocellulose in freshly prepared PBS containing 3% nonfat dry milk (Catalog # 20-200), (PBS-MLK) for 90 minutes at room temperature with constant agitation.
4. Incubate the nitrocellulose with **0.5-2 μ g/mL of anti-TrkB**, diluted in freshly prepared PBS-MLK overnight with agitation at 4°C.
5. Wash the nitrocellulose twice with water.
6. Incubate the nitrocellulose in the secondary reagent of choice (a goat anti-rabbit HRP conjugated IgG, Catalog # 12-348, 1:5000 dilution was used) in PBS-MLK for 1.5 hours at room temperature with agitation.
7. Wash the nitrocellulose with water twice.
8. Wash the nitrocellulose in PBS-0.05% Tween 20 for 3-5 minutes.
9. Rinse the nitrocellulose in 4-5 changes of water.
10. Use detection method of choice (enhanced chemiluminescence was used).