



cell signaling solutions

## Certificate of Analysis

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### Anti-TrkB

(rabbit polyclonal IgG)

Catalog # 07-225

Lot # 28846

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

**Species Cross-reactivity:** Rat and mouse.

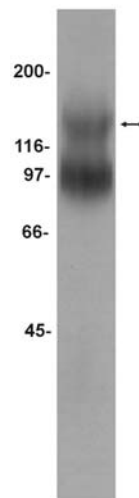
**Formulation:** 200 $\mu$ g of protein A purified rabbit IgG in 200 $\mu$ l of 0.1M Tris-glycine, pH 7.4, 0.15M NaCl, 0.05% sodium azide before the addition of glycerol to 30%. Liquid at -20°C.

**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 $\mu$ g/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 $\mu$ g/ml). Proteins were visualized using a goat anti-rabbit secondary antibody conjugated to HRP and a chemiluminescence detection system. Arrow indicates TrkB (~145kDa).

### General References:

1. Weskamp, G., and L.F. Reichardt, *Neuron* **6**: 649-663, 1991.
2. Clary, D.O., *et al.*, *Mol. Biol. Cell* **5**: 549-563, 1994.
3. Huang, E.J., *et al.*, *Development* **126**: 2191-2203, 1999.

### Immunoblot Protocol

1. Perform SDS-polyacrylamide gel electrophoresis (SDS-PAGE) on a cell lysate sample (cell lysis buffer: 50mM Tris-HCl, pH 7.4; 1% NP-40; 0.25% sodium deoxycholate; 150mM NaCl; 1mM EGTA; 1mM PMSF; 1 $\mu$ g/ml each aprotinin, leupeptin, pepstatin; 1mM Na<sub>3</sub>VO<sub>4</sub>; 1mM 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 $\mu$ 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).