# Rabbit (polyclonal) Anti-TrkA [pY$^{751}$] Phosphospecific Antibody, Unconjugated

## PRODUCT ANALYSIS SHEET

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|---|---|
| **Catalog Number:** | 44-1342G (10 mini-blot size) |
| **Lot Number:** | See product label |
| **Volume:** | 100 µL |
| **Form of Antibody:** | Rabbit polyclonal immunoglobulin in Dulbecco's phosphate buffered saline (without $Mg^{2+}$ and $Ca^{2+}$), pH 7.3 (+/- 0.1), 50% glycerol, with 1.0 mg/mL BSA (IgG, protease free) as a carrier. |
| **Preservative:** | 0.05% sodium azide (Caution: sodium azide is a poisonous and hazardous substance. Handle with care and dispose of properly.) |
| **Purification:** | Purified from rabbit serum by sequential epitope-specific chromatography. The antibody has been negatively pre-adsorbed using a non-phosphopeptide corresponding to the site of phosphorylation to remove antibody that is reactive with non-phosphorylated TrkA. The final product is generated by affinity chromatography using a TrkA-derived peptide that is phosphorylated at tyrosine 751. |
| **Immunogen:** | The antiserum was produced against a chemically synthesized phosphopeptide derived from the region of human TrkA that contains tyrosine 751. |
| **Target Summary:** | TrkA (also known as NTRK1) is a 140 kDa member of the Trk family of receptor tyrosine kinases that plays an important role in survival and differentiation and is implicated in sensory perception and cognitive behavior. Deregulation of Trk activities is associated with numerous human disorders including neuroblastoma and congenital insensitivity to pain with anhidrosis (CIPA). NGF and other neutrophins initiate signaling through TrkA by the sequential dimerization, trans-autophosphorylation on tyrosine residues and kinase activation, followed by binding and tyrosine phosphorylation of substrates. TrkA is phosphorylated on several tyrosine sites including tyrosines 490, 670, 674, 675, 751, and 785. Tyrosine 751 is an autophosphorylation site located in the cytoplasmic domain and is shown to mediate TrkA binding to the p85 subunit of phosphatidylinositol 3'-kinase. |
| **Reactivity:** | Human and rat TrkA. This antibody may also detect phosphorylated TrkB (80% homology). |
| **Applications:** | The antibody has been used for Western blotting applications. |
| **Suggested Working Dilutions:** | For Western blotting applications, we recommend using the antibody at a 1:1000 dilution. The optimal antibody concentration should be determined empirically for each specific application. |
| **Storage:** | Store at –20°C. We recommend a brief centrifugation before opening to settle vial contents. Then, apportion into working aliquots and store at –20°C. For shipment or short-term storage (up to one week), 2-8°C is sufficient. |
| **Expiration Date:** | Expires one year from date of receipt when stored as instructed. |
| **Positive Controls Used:** | Rat pheochromocytoma (PC12) cells stimulated with nerve growth factor (NGF). |



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**Related Products:**

**Antibodies:**

TrkA [pYpY$^{674/675}$], Cat. # 44-1339G
TrkA [pYpYpY$^{670/674/675}$], Cat. # 44-1340G
c-Ret [pY$^{1016}$], Cat. # 44-277G
c-Ret [pS$^{696}$], Cat. # 44-274G
FAK [pY$^{397}$], Cat. # 44-624G
FAK [pY$^{576}$], Cat. # 44-652G
Erk1/2 [pTpY$^{185/187}$], Cat. # 44-680G
Shc [pYpY$^{239/240}$], Cat.# 44-830G
PLCγ [pY$^{783}$], Cat.# 44-696G
Src [pY$^{418}$], Cat. # 44-660G
SHP2 [pY$^{542}$], Cat. # 44-554
STAT3 [pY$^{705}$], Cat.# 44-380G
RanBP3 [pS$^{58}$], Cat. # 44-1180G
Integrin β1 [pTpT$^{788/789}$], Cat. # 44-872
Integrin β3 [pY$^{773}$], Cat. # 44-876
Integrin β3 [pY$^{785}$], Cat. # 44-878
VE-cadherin [pY$^{731}$], Cat. # 44-1145G

**References:**


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Arevalo, J.C., et al. (2006) Identification of a switch in neurotrophin signaling by selective tyrosine phosphorylation. J. Biol. Chem. 281(2):1001-1007.

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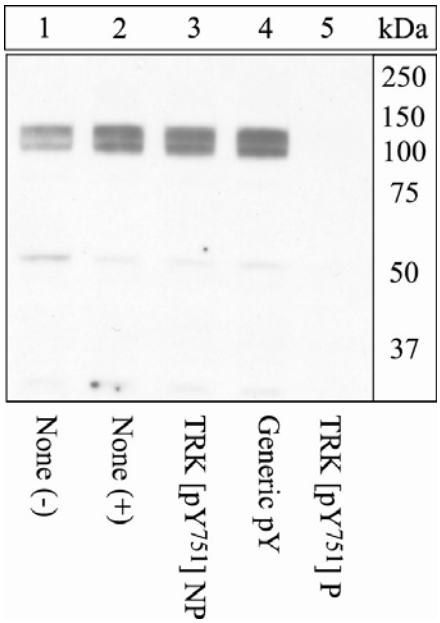


**Antibody Specificity**

Lysates prepared from unstimulated (lane 1) or NGF-treated PC12 cells (lanes 2-5) were resolved on an 8% polyacrylamide gel and transferred to PVDF. The membrane was blocked with a 3% BSA-TBST buffer for one hour at room temperature, and then incubated with the TrkA [pY$^{751}$] antibody for two hours at room temperature in 3% BSA-TBST buffer, following prior incubation with: no peptide (lanes 1 and 2), the non-phosphopeptide corresponding to the phosphopeptide immunogen (3), a generic phosphotyrosine-containing peptide (4), or the phosphopeptide immunogen corresponding to TrkA [pY$^{751}$] (5). After washing, the membrane was incubated with goat F(ab')$_2$ anti-rabbit IgG HRP conjugate (Cat. # ALI4404) and signals were detected using the Pierce SuperSignal™ reagent.

The data show that the signal was selectively blocked by the phosphopeptide corresponding to TrkA [pY$^{751}$] indicating that the signal is phosphorylation site-specific.




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**Western Blotting Procedure**

1. Lyse approximately $10^7$ cells in 0.5 mL of ice cold Cell Lysis Buffer (formulation provided below). This buffer, a modified RIPA buffer, is suitable for recovery of most proteins, including membrane receptors, cytoskeletal-associated proteins, and soluble proteins. This cell lysis buffer formulation is available as a separate product which requires supplementation with protease inhibitors immediately prior to use (Cat. # FNN0011). Other cell lysis buffer formulations, such as Laemmli sample buffer and Triton-X 100 buffer, are also compatible with this procedure. Additional optimization of the cell stimulation protocol and cell lysis procedure may be required for each specific application.
2. Remove the cellular debris by centrifuging the lysates at 14,000 x g for 10 minutes. Alternatively, lysates may be ultracentrifuged at 100,000 x g for 30 minutes for greater clarification.
3. Carefully decant the clarified cell lysates into clean tubes and determine the protein concentration using a suitable method, such as the Bradford assay. Polypropylene tubes are recommended for storing cell lysates.
4. React an aliquot of the lysate with an equal volume of 2x Laemmli Sample Buffer (125 mM Tris, pH 6.8, 10% glycerol, 10% SDS, 0.006% bromophenol blue, and 130 mM dithiothreitol [DTT]) and boil the mixture for 90 seconds at 100°C.
5. Load 10-30 µg of the cell lysate into the wells of an appropriate single percentage or gradient minigel and resolve the proteins by SDS-PAGE.
6. In preparation for the Western transfer, cut a piece of PVDF membrane slightly larger than the gel. Soak the membrane in methanol for 1 minute, then rinse with $ddH_2O$ for 5 minutes. Alternatively, nitrocellulose may be used.
7. Soak the membrane, 2 pieces of Whatman paper, and Western apparatus sponges in transfer buffer (formulation provided below) for 2 minutes.
8. Assemble the gel and membrane into the sandwich apparatus.
9. Transfer the proteins at 140 mA for 60-90 minutes at room temperature.
10. Following the transfer, rinse the membrane with Tris buffered saline for 2 minutes.
11. Block the membrane with blocking buffer (formulation provided below) for one hour at room temperature or overnight at 4°C.
12. Incubate the blocked blot with primary antibody at a 1:1000 dilution in Tris buffered saline supplemented with 3% Ig-free BSA and 0.1% Tween 20 overnight at 4°C or for one hour at room temperature.
13. Wash the blot with several changes of Tris buffered saline supplemented with 0.1% Tween 20.
14. Detect the antibody band using an appropriate secondary antibody, such as goat $F(ab')_2$ anti-rabbit IgG alkaline phosphatase conjugate (Cat. # ALI4405) or goat $F(ab')_2$ anti-rabbit IgG horseradish peroxidase conjugate (Cat. # ALI4404) in conjunction with your chemiluminescence reagents and instrumentation.

**Cell Lysis Buffer Formulation:**
10 mM Tris, pH 7.4
100 mM NaCl
1 mM EDTA
1 mM EGTA
1 mM NaF
20 mM $Na_4P_2O_7$
2 mM $Na_3VO_4$
0.1% SDS
0.5% sodium deoxycholate
1% Triton-X 100
10% glycerol
1 mM PMSF (made from a 0.3 M stock in DMSO) or 1 mM AEBSF (water soluble version of PMSF)
60 µg/mL aprotinin
10 µg/mL leupeptin
1 µg/mL pepstatin
(alternatively, protease inhibitor cocktail such as Sigma Cat. # P2714 may be used)

**Transfer Buffer Formulation:**
2.4 gm Tris base
14.2 gm glycine
200 mL methanol
Q.S. to 1 liter, then add
1 mL 10% SDS.
Cool to 4°C prior to use.

**Tris Buffered Saline Formulation:**
20 mM Tris-HCl, pH 7.4
0.9% NaCl

**Blocking Buffer Formulation:**
100 mL Tris buffered saline
3 gm Ig-free BSA
0.1 mL Tween 20



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