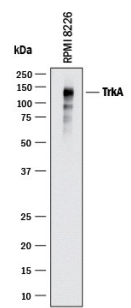


| DESCRIPTION               |   |
|---------------------------|---|
| <b>Species Reactivity</b> | Human   |
| <b>Specificity</b>        | Detects human TrkA in direct ELISAs and Western blots. In direct ELISAs, approximately 50% cross-reactivity with recombinant rat TrkA is observed and 10% cross-reactivity with recombinant human (rh) TrkB and rhTrkC is observed. |
| <b>Source</b>             | Polyclonal Goat IgG   |
| <b>Purification</b>       | Antigen Affinity-purified   |
| <b>Immunogen</b>          | Mouse myeloma cell line NS0-derived recombinant human TrkA<br>Ala33-Glu407<br>Accession # AAA36770  |
| <b>Endotoxin Level</b>    | <0.10 EU per 1 µg of the antibody by the LAL method.  |
| <b>Formulation</b>        | Lyophilized from a 0.2 µm filtered solution in PBS with Trehalose. See Certificate of Analysis for details.<br>*Small pack size (-SP) is supplied as a 0.2 µm filtered solution in PBS.   |

| APPLICATIONS   |   |
|--|---|
| <b>Please Note:</b> Optimal dilutions should be determined by each laboratory for each application. <i>General Protocols</i> are available in the <i>Technical Information</i> section on our website. |   |
|  | <b>Recommended Concentration      Sample</b>  |
| <b>Western Blot</b>  | 1 µg/mL      See Below  |
| <b>Flow Cytometry</b>  | 2.5 µg/10 <sup>6</sup> cells      K562 human chronic myelogenous leukemia cell line   |
| <b>Immunohistochemistry</b>  | 5-15 µg/mL      See Below   |
| <b>CyTOF-ready</b>   | Ready to be labeled using established conjugation methods. No BSA or other carrier proteins that could interfere with conjugation.  |
| <b>Neutralization</b>  | Measured by its ability to neutralize β-NGF-induced proliferation in the TF-1 human erythroleukemic cell line. Kitamura, T. <i>et al.</i> (1989) <i>J. Cell Physiol.</i> <b>140</b> :323. The Neutralization Dose (ND <sub>50</sub> ) is typically 3-12 µg/mL in the presence of 5 ng/mL Recombinant Human β-NGF. |

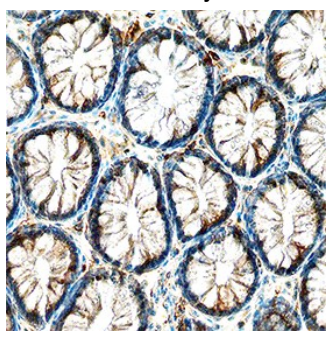
**DATA**

**Western Blot**



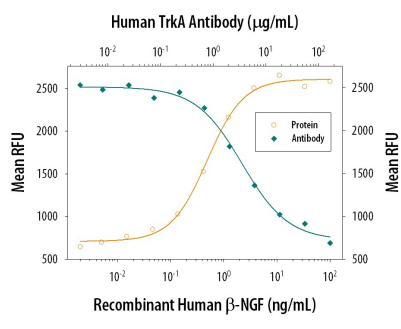
**Detection of Human TrkA by Western Blot.** Western blot shows lysates of RPMI 8226 human multiple myeloma cell line. PVDF membrane was probed with 1 µg/mL of Goat Anti-Human TrkA Antigen Affinity-purified Polyclonal Antibody (Catalog # AF175) followed by HRP-conjugated Anti-Goat IgG Secondary Antibody (Catalog # HAF017). A specific band was detected for TrkA at approximately 140 kDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.

**Immunohistochemistry**



**TrkA in Human Colon.** TrkA was detected in immersion fixed paraffin-embedded sections of human colon using Goat Anti-Human TrkA Antigen Affinity-purified Polyclonal Antibody (Catalog # AF175) at 3 µg/mL overnight at 4 °C. Before incubation with the primary antibody, tissue was subjected to heat-induced epitope retrieval using Antigen Retrieval Reagent-Basic (Catalog # CTS013). Tissue was stained using the Anti-Goat HRP-DAB Cell & Tissue Staining Kit (brown; Catalog # CTS008) and counterstained with hematoxylin (blue). Specific staining was localized to cytoplasm in colon glands. View our protocol for [Chromogenic IHC Staining of Paraffin-embedded Tissue Sections](#).

**Neutralization**



**Cell Proliferation Induced by β-NGF and Neutralization by Human TrkA Antibody.** Recombinant Human β-NGF (Catalog # 256-GF) stimulates proliferation in the TF-1 human erythroleukemic cell line in a dose-dependent manner (orange line). Proliferation elicited by Recombinant Human β-NGF (5 ng/mL) is neutralized (green line) by increasing concentrations of Goat Anti-Human TrkA Antigen Affinity-purified Polyclonal Antibody (Catalog # AF175). The ND<sub>50</sub> is typically 3-12 µg/mL.

#### PREPARATION AND STORAGE

|                                |  |
|--------------------------------|--|
| <b>Reconstitution</b>          | Reconstitute at 0.2 mg/mL in sterile PBS.  |
| <b>Shipping</b>                | The product is shipped at ambient temperature. Upon receipt, store it immediately at the temperature recommended below.<br>*Small pack size (-SP) is shipped with polar packs. Upon receipt, store it immediately at -20 to -70 °C   |
| <b>Stability &amp; Storage</b> | <b>Use a manual defrost freezer and avoid repeated freeze-thaw cycles.</b> <ul style="list-style-type: none"><li>● 12 months from date of receipt, -20 to -70 °C as supplied.</li><li>● 1 month, 2 to 8 °C under sterile conditions after reconstitution.</li><li>● 6 months, -20 to -70 °C under sterile conditions after reconstitution.</li></ul> |

#### BACKGROUND

TrkA, the product of the proto-oncogene *trk*, is a member of the neurotrophic tyrosine kinase receptor family that has three members. TrkA, TrkB and TrkC preferentially bind NGF, NT-4 and BDNF, and NT-3, respectively. All Trk family proteins share a conserved complex subdomain organization consisting of a signal peptide, two cysteine-rich domains, a cluster of three leucine-rich motifs, and two immunoglobulin-like domains in the extracellular region, as well as an intracellular region that contains the tyrosine kinase domain. Two distinct TrkA isoforms that differ by virtue of a 6-amino acid insertion in their extracellular domain have been identified. The longer TrkA isoform is the only isoform expressed within neuronal tissues whereas the shorter TrkA is expressed mainly in non-neuronal tissues. NGF binds to TrkA with low affinity and activates its cytoplasmic kinase, initiating a signaling cascade that mediates neuronal survival and differentiation. Higher affinity binding of NGF requires the coexpression of TrkA with the p75 NGF receptor (NGFR), a member of the tumor necrosis factor receptor superfamily. NGFR binds all neurotrophins with low affinity and modulates Trk activity as well as alters the specificity of Trk receptors for their ligands. NGFR can also mediate cell death when expressed independent of Trk.

#### References:

1. Esposito, D. *et al.* (2001) *J. Biol. Chem.* **276**:32687.
2. Sofroniew, M.V. *et al.* (200) *Annu. Rev. Neurosci.* **24**:1217.