

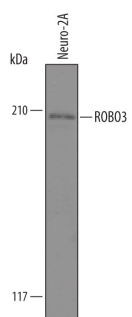
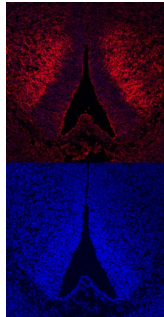
DESCRIPTION	
Species Reactivity	Mouse
Specificity	Detects mouse ROBO3 in direct ELISAs and Western blots. In direct ELISAs, approximately 40% cross-reactivity with recombinant human (rh) ROBO3 and less than 5% cross-reactivity with rhROBO2 and rhROBO4 is observed.
Source	Polyclonal Goat IgG
Purification	Antigen Affinity-purified
Immunogen	Mouse myeloma cell line NS0-derived mouse ROBO3 Gly54-Ser545 Accession # Q9Z2I4
Formulation	Lyophilized from a 0.2 µm filtered solution in PBS with Trehalose. See Certificate of Analysis for details. *Small pack size (-SP) is supplied either lyophilized or as a 0.2 µm filtered solution in PBS.

APPLICATIONS

Please Note: Optimal dilutions should be determined by each laboratory for each application. *General Protocols* are available in the *Technical Information* section on our website.

	Recommended Concentration	Sample
Western Blot	0.5 µg/mL	See Below
Immunohistochemistry	5-15 µg/mL	See Below

DATA

<p>Western Blot</p>  <p>Detection of Mouse ROBO3 by Western Blot. Western blot shows lysates of Neuro-2A mouse neuroblastoma cell line. PVDF Membrane was probed with 0.5 µg/mL of Mouse ROBO3 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF3155) followed by HRP-conjugated Anti-Goat IgG Secondary Antibody (Catalog # HAF019). A specific band was detected for ROBO3 at approximately 205 kDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.</p>	<p>Immunohistochemistry</p>  <p>ROBO3 in Mouse Embryo. ROBO3 was detected in immersion fixed frozen sections of mouse embryo (E11.5) using Mouse ROBO3 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF3155) at 25 µg/mL overnight at 4 °C. Tissue was stained using the NorthernLights™ 557-conjugated Anti-Goat IgG Secondary Antibody (red, upper panel; Catalog # NL001) and counterstained with DAPI (blue, lower panel). Specific staining was localized to developing forebrain. View our protocol for Fluorescent IHC Staining of Frozen Tissue Sections.</p>
--	--

PREPARATION AND STORAGE

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

BACKGROUND

Mouse ROBO3 (also named Rig-1) is a 200 kDa member of the ROBO family of guidance molecules (1-3). The term ROBO derives from round-about, a description of the circuitous pathway axons take in the absence of a functional ROBO gene (3, 4). Mouse ROBO3 is a type I transmembrane glycoprotein that is synthesized as a 1366 amino acid (aa) precursor. It contains a 20 aa signal sequence, an 871 aa extracellular domain (ECD), a 21 aa transmembrane segment, and a 454 aa cytoplasmic region (5, 6). The ECD contains five C2-type Ig-like domains (aa 64-531) and three fibronectin (FN) type III domains (aa 555-863). The cytoplasmic region contains three of four possible 15-20 aa long CC (conserved cytoplasmic) motifs that are found in ROBO-1 (7, 8). Mouse ROBO3 has multiple isoforms. An alternate start site generates an 1366 aa (precursor) A isoform and a 1344 aa (precursor) B isoform. These two forms only differ in the first 53 and 31 amino acids of the precursor, respectively (9). There are reportedly nine splice variants in the mouse ROBO3 gene. Three result in soluble forms. Little information exists about the isoforms. Mouse ROBO3 ECD is 95%, 84% and 86% aa identical to the ROBO3 ECD in rat, human and canine, respectively. Normally, axons originating on one side of the spinal cord are inhibited from crossing to the other side by a SLIT2-ROBO1 interaction at the midline. ROBO3 is permissive for this event. It is unclear how this is accomplished. One possibility is that it binds directly to ROBO-1, blocking SLIT activation. A second possibility involves ROBO3 binding to SLIT2 in a nonproductive interaction. In human, only ROBO3 Form B is known to bind to SLIT2 (9-11).

References:

1. Rajagopalan, S. *et al.* (2000) *Neuron* **28**:767.
2. Guthrie, S. (2004) *Curr. Biol.* **14**:R632.
3. Guthrie, S. (2001) *Curr. Biol.* **11**:R300.
4. Seeger, M. *et al.* (1993) *Neuron* **10**:409.
5. Yuan, S-S.F. *et al.* (1999) *Dev. Biol.* **207**:62.
6. SwissProt. Accession # Q9Z2I4.
7. Bashaw, G.J. *et al.* (2000) *Cell* **101**:703.
8. Kidd, T. *et al.* (1998) *Cell* **92**:205.
9. Camurri, L. *et al.* (2005) *Mol. Cell. Neurosci.* **30**:485.
10. Sabatier, C. *et al.* (2004) *Cell* **117**:157.
11. Mambetisaeva, E.T. *et al.* (2005) *Dev. Dyn.* **233**:41.