

DESCRIPTION	
Species Reactivity	Human
Specificity	Detects ROBO3 in direct ELISAs and Western blots. In direct ELISAs and Western blots, approximately 60% cross-reactivity with recombinant mouse ROBO3 is observed, 5% cross-reactivity with recombinant human ROBO2 and recombinant rat ROBO1 is observed, and less than 1% cross-reactivity with recombinant human ROBO4 is observed.
Source	Polyclonal Goat IgG
Purification	Antigen Affinity-purified
Immunogen	Mouse myeloma cell line NS0-derived recombinant human ROBO3 Leu40-Ser545 Accession # Q96MS0
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	0.1 µg/mL	Recombinant Human ROBO3 Fc Chimera (Catalog # 3076-RB)
Immunohistochemistry	5-15 µg/mL	Immersion fixed frozen sections of embryonic mouse spinal cord (E11.5)

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

Human ROBO3 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). Human ROBO3 is a type I transmembrane glycoprotein that is synthesized as a 1386 amino acid (aa) precursor. It contains a 20 aa signal sequence, an 871 aa extracellular domain (ECD), a 21 aa transmembrane segment, and a 474 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 ROBO1 (7, 8). Human ROBO3 has multiple isoforms. An alternate start site generates a 1365 aa A isoform and a 1341 aa B isoform. These two mature forms differ only over the first 26 and 2 amino acids of the N-terminus, respectively. There are multiple point mutations and insertions in the ROBO3 gene. Three result in truncated forms. One is 456 aa in length and ends after the Ig-like domain # 4. A second is 770 aa in length and ends in the third FN domain. A third isoform is truncated after aa 1108 in the cytoplasmic region after CC2. At least one alternate splice form is also reported. It shows a 10 aa substitution between aa 1025-1034, followed by truncation. Human ROBO3 ECD is 84% and 91% aa identical to the ECD in mouse and canine ROBO3, 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 ROBO1, blocking SLIT activation. A second possibility involves ROBO3 binding to SLIT2 in a nonproductive interaction. However, only ROBO3 Form B is known to bind to SLIT2 (9-11).

References:

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