

DESCRIPTION

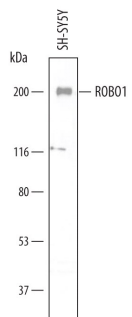
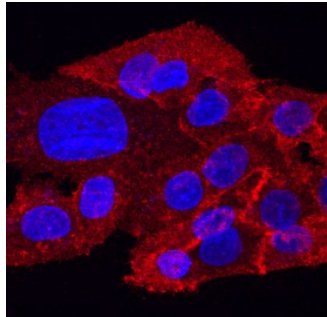
Species Reactivity	Human
Specificity	Detects human ROBO1 in direct ELISAs and Western blots. In direct ELISAs, approximately 10% cross-reactivity with recombinant rat ROBO1 is observed, and less than 3% cross-reactivity with recombinant human (rh) ROBO2 and rhROBO3 is observed.
Source	Polyclonal Sheep IgG
Purification	Antigen Affinity-purified
Immunogen	Mouse myeloma cell line NS0-derived recombinant human ROBO1 Ser20-Pro861 (Val310-Ser312 del) Accession # NP_598334
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 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	1 µg/mL	See Below
Immunocytochemistry	5-15 µg/mL	See Below

DATA

<p>Western Blot</p>  <p>Detection of Human ROBO1 by Western Blot. Western blot shows lysates of SH-SY5Y human neuroblastoma cell line. PVDF membrane was probed with 1 µg/mL of Sheep Anti-Human ROBO1 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF7118) followed by HRP-conjugated Anti-Sheep IgG Secondary Antibody (Catalog # HAF016). A specific band was detected for ROBO1 at approximately 200 kDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.</p>	<p>Immunocytochemistry</p>  <p>ROBO1 in HepG2 Human Cell Line. ROBO1 was detected in immersion fixed HepG2 human hepatocellular carcinoma cell line using Sheep Anti-Human ROBO1 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF7118) at 10 µg/mL for 3 hours at room temperature. Cells were stained using the NorthernLights™ 557-conjugated Anti-Sheep IgG Secondary Antibody (red; Catalog # NL010) and counterstained with DAPI (blue). Specific staining was localized to cell surfaces and cytoplasm. View our protocol for Fluorescent ICC Staining of Cells on Coverslips.</p>
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PREPARATION AND STORAGE

Reconstitution	Sterile PBS to a final concentration of 0.2 mg/mL.
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

ROBO1 (Roundabout-like protein 1) is a 190-230 kDa member of the ROBO/roundabout receptor family. It is expressed by commissural axons from multiple nuclei, and is also found on vascular endothelium, bronchial epithelium, and synctiotrophoblasts. It interacts with Slit and DCC to temporally regulate the migration of axonal processes. The human ROBO1 precursor is a 1651 amino acid (aa) type I transmembrane protein. It contains a 25 aa signal sequence, followed by an 872 aa extracellular region (aa 26-897) that possesses five C2-type Ig-like domains (aa 68-541) and three fibronectin type III domains (aa 561-864) (SwissProt # Q9Y6N7). ROBO1 shows multiple isoform variants. The variant used here is termed ROBO1b/DUTT1 (Genbank # NP_598334), and it possesses an 18 aa substitution for aa 1-47, accompanied by a three aa insertion after Gln348, and a deletion of aa 939-947. ROBO1a, by contrast, possesses only the 18 aa substitution just described. A third isoform possesses the same changes as ROBO1b plus an additional deletion of aa 1013-1067, while a final variant utilizes an alternative start site at Met120. Proteolytic cleavage generates a soluble 120 kDa N-terminal fragment. Over aa 20-861, human ROBO1b shares 97% aa identity with mouse ROBO1.