Human/Mouse Neogenin Antibody

Antigen Affinity-purified Polyclonal Goat IgG
Catalog Number: AF1079

DESCRIPTION

Species Reactivity	Human/Mouse
Specificity	Detects human and mouse Neogenin in direct ELISAs and Western blots. In Western blots, less than 5% cross-reactivity with recombinant mouse DCC is observed.
Source	Polyclonal Goat IgG
Purification	Antigen Affinity-purified
Immunogen	Mouse myeloma cell line NS0-derived recombinant mouse Neogenin Ala42-Ile1033 (Asp442-Leu461 del) Accession # NP_032710
Endotoxin Level	<0.10 EU per 1 µg of the antibody by the LAL method.
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.

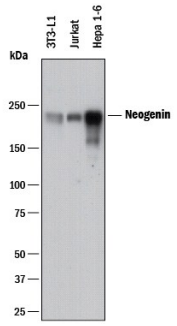
APPLICATIONS

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	0.5 µg/mL	See Below
Immunohistochemistry	5-15 µg/mL	See Below
Simple Western	10 µg/mL	See Below
Blockade of Receptor-ligand Interaction	In a functional ELISA, 2.5-10 µg/mL of this antibody will block 50% of the binding of 200 ng/mL of Recombinant Chicken Netrin-1 (Catalog # 128-N1) to immobilized Recombinant Mouse Neogenin Fc Chimera (Catalog # 1079-NE) coated at 5 µg/mL (100 µL/well). At 300 µg/mL, this antibody will block >90% of the binding.	

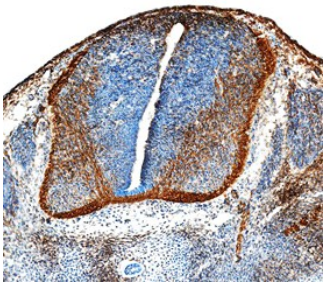
DATA

Western Blot



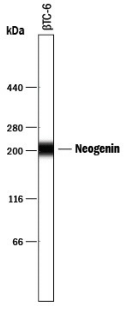
Detection of Human and Mouse Neogenin by Western Blot. Western blot shows lysates of 3T3-L1 mouse embryonic fibroblast adipose-like cell line, Jurkat human acute T cell leukemia cell line, and Hepa 1-6 mouse hepatoma cell line. PVDF membrane was probed with 0.5 µg/mL of Goat Anti-Human/Mouse Neogenin Antigen Affinity-purified Polyclonal Antibody (Catalog # AF1079) followed by HRP-conjugated Anti-Goat IgG Secondary Antibody (Catalog # HAF017). A specific band was detected for Neogenin at approximately 230 kDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.

Immunohistochemistry



Neogenin in Mouse Embryo. Neogenin was detected in perfusion fixed frozen sections of mouse embryo (15 d.p.c.) using Goat Anti-Human/Mouse Neogenin Antigen Affinity-purified Polyclonal Antibody (Catalog # AF1079) at 15 µg/mL overnight at 4 °C. 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 developing neurons. View our protocol for Chromogenic IHC Staining of Frozen Tissue Sections.

Simple Western



Detection of Mouse Neogenin by Simple Western™. Simple Western lane view shows lysates of βTC-6 mouse beta cell insulinoma cell line, loaded at 0.2 mg/mL. A specific band was detected for Neogenin at approximately 207 kDa (as indicated) using 10 µg/mL of Goat Anti-Human/Mouse Neogenin Antigen Affinity-purified Polyclonal Antibody (Catalog # AF1079) followed by 1:50 dilution of HRP-conjugated Anti-Goat IgG Secondary Antibody (Catalog # HAF109). This experiment was conducted under reducing conditions and using the 12-230 kDa separation system.

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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.** ● 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

Neogenin (NEO) is a type I transmembrane protein that is crucial for axonal guidance and neuronal migration. It is also involved in regulating differentiation programs in many embryonic and adult tissues (1). Mouse NEO is widely expressed in adult tissues and is expressed throughout the mid to late stages of gestation, in both neuronal and non-neuronal tissues. It is a member of the immunoglobulin (Ig) superfamily and is closely related to deleted in colorectal cancer (DCC). Mouse NEO cDNA encodes a 1493 amino acid residue (aa) precursor with a putative 36 aa signal peptide, a 1100 aa extracellular domain with six Ig-like C2 type domains and three fibronectin type III domains, a 21 aa transmembrane domain, and a 345 aa cytoplasmic domain. At least five isoforms are produced in mice by alternative splicing. Mouse NEO shares 96%, 93%, and 86% aa sequence identity with rat, human, and chicken NEO, respectively. It also has 46% and 29% sequence homology with mouse DCC and *C. elegans* UNC40, a homolog of DCC. NEO and DCC, together with the UNC5 family of type I transmembrane proteins, are receptors for the netrin/UNC6 family of laminin-related bifunctional guidance molecules that both attract some axons and repel others (2, 3). In mouse, at least five netrins (netrin-1, -3, -4, G1, and G2) have been identified (3-5). Mouse netrin-1 and netrin-3 have been shown to be ligands for mouse NEO.

References:

1. Keeling, S.L. *et al.* (1997) Oncogene **15**:691.
2. Hong, K. *et al.* (1999) Cell **97**:927.
3. Livesey, F.J. (1999) Cell Mol. Life Sci. **56**:62.
4. Nakashiba, T. *et al.* (2000) J. Neurosci. **20**:6540.
5. Nakashiba, T. *et al.* (2002) Mech. Dev. **111**:47.

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