

DESCRIPTION

Species Reactivity	Human/Mouse/Rat
Specificity	Detects human, mouse, and rat Semaphorin 5A in direct ELISAs and Western blots. In direct ELISAs, less than 1% cross-reactivity with recombinant human (rh) Sema3B, rhSema4C, and rhSema6A is observed.
Source	Polyclonal Sheep IgG
Purification	Antigen Affinity-purified
Immunogen	Chinese hamster ovary cell line CHO-derived recombinant human Semaphorin 5A Glu23-Thr765 Accession # AAC09473
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.

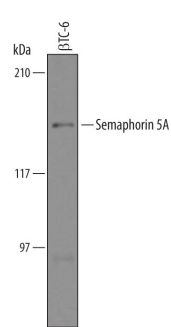
APPLICATIONS

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
Immunohistochemistry	5-15 µg/mL	See Below

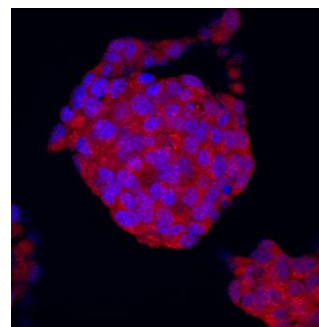
DATA

Western Blot



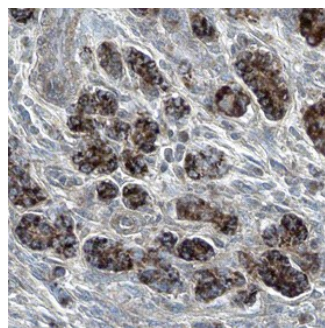
Detection of Mouse Semaphorin 5A by Western Blot. Western blot shows lysates of β TC-6 mouse beta cell insulinoma cell line. PVDF Membrane was probed with 1 µg/mL of Sheep Anti-Human/Mouse/Rat Semaphorin 5A Antigen Affinity-purified Polyclonal Antibody (Catalog # AF5896) followed by HRP-conjugated Anti-Sheep IgG Secondary Antibody (Catalog # HAF016). A specific band was detected for Semaphorin 5A at approximately 135 kDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 8.

Immunocytochemistry



Semaphorin 5A in β TC-6 Mouse Cell Line. Semaphorin 5A was detected in immersion fixed β TC-6 mouse beta cell insulinoma cell line using Sheep Anti-Human/Mouse/Rat Semaphorin 5A Antigen Affinity-purified Polyclonal Antibody (Catalog # AF5896) 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 cytoplasm. View our protocol for [Fluorescent ICC Staining of Cells on Coverslips](#).

Immunohistochemistry



Semaphorin 5A in Human Pancreas. Semaphorin 5A was detected in immersion fixed paraffin-embedded sections of human pancreas using Sheep Anti-Human/Mouse/Rat Semaphorin 5A Antigen Affinity-purified Polyclonal Antibody (Catalog # AF5896) at 5 µg/mL overnight at 4 °C. Tissue was stained using the Anti-Sheep HRP-DAB Cell & Tissue Staining Kit (brown; Catalog # CTS019) and counterstained with hematoxylin (blue). Specific staining was localized to the plasma membranes of epithelial cells. View our protocol for [Chromogenic IHC Staining of Paraffin-embedded Tissue Sections](#).

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

Semaphorin 5A (Sema5A, previously called SemaF) is a 140 kDa protein of the semaphorin family of axon guidance molecules (1-4). Class 5 semaphorins are type I transmembrane glycoproteins with an N-terminal Sema domain and multiple juxtamembrane type 1 thrombospondin (TSP) repeats within their extracellular domains (1-3). Sema5A is expressed developmentally in oligodendrocytes, neuroepithelial cells surrounding retinal axons, the base of limb buds, the cardiac atrial septum and endocardial cushions, and the mesoderm surrounding cranial vessels (4-6). The human Sema5A cDNA encodes a 22 amino acid (aa) signal sequence, a 946 aa extracellular domain (ECD), a 22 aa transmembrane sequence and an 85 aa cytoplasmic portion. Within aa 23-765, which includes the sema domain and four of the seven TSP repeats, human Sema5A shares 93% aa identity with corresponding mouse, rat, and canine sequences. Semaphorins typically transduce signals through transmembrane plexins (1, 2). The sema domain of Sema5A binds plexin B3, triggering signaling via HGF R/c-Met (7). Both Sema5A and plexin B3 are expressed postnatally during differentiation and migration of central nervous system oligodendrocytes. However, plexin B3 is not significantly expressed prenatally and therefore unlikely to be the Sema5A receptor during development (7, 8). The Sema5A TSP repeats interact with either heparin sulfate or chondroitin sulfate proteoglycans (HSPG, CSPG) (9). HSPG interaction promotes attraction, while CSPG interaction promotes repulsion and is essential for axon fasciculation, independent of plexin B3 (9, 10). Sema5A mutations have been implicated in the genetic syndrome, cri-du-chat, while some polymorphisms may increase risk for neurodegenerative diseases such as Parkinson's (3, 11). Sema5A expression may be upregulated in metastatic cancer cells and downregulated in autism (12, 13).

References:

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