

DESCRIPTION

Species Reactivity	Human/Mouse/Rat
Specificity	Detects human, mouse, and rat SNAP25 in direct ELISAs and Western blots. In direct ELISAs, approximately 5% cross-reactivity with recombinant human SNAP23 is observed.
Source	Polyclonal Sheep IgG
Purification	Antigen Affinity-purified
Immunogen	<i>E. coli</i> -derived recombinant human SNAP25 Asp140-Gly206 Accession # P60880
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.

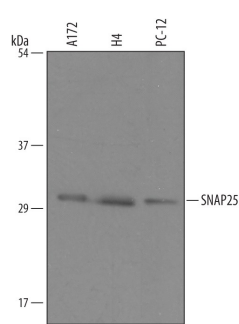
APPLICATIONS

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

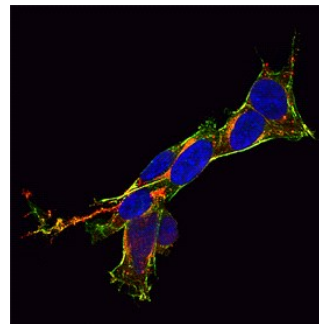
DATA

Western Blot



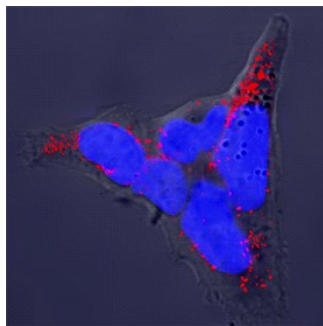
Detection of Human and Rat SNAP25 by Western Blot. Western blot shows lysates of A172 human glioblastoma cell line, H4 human neuroglioma cell line, and PC-12 rat adrenal pheochromocytoma cell line. PVDF Membrane was probed with 1 µg/mL of Sheep Anti-Human/Mouse/Rat SNAP25 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF5946) followed by HRP-conjugated Anti-Sheep IgG Secondary Antibody (Catalog # HAF016). A specific band was detected for SNAP25 at approximately 29 kDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 8.

Immunocytochemistry



SNAP25 in SH-SY5Y Human Cell Line. SH-SY5Y human neuroblastoma cells were cultured overnight in the presence of 1 mM Retinoic Acid (Catalog # 069550) prior to immersion fixation. SNAP25 was detected using a Sheep Anti-Human/Mouse/Rat SNAP25 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF5946). The cells were stained with the NorthernLights 557-conjugated Donkey Anti-Sheep Affinity-purified IgG Secondary Antibody (red; Catalog # NL010). Actin filaments were stained with FITC-conjugated Phalloidin (green) and cell nuclei were counterstained with DAPI (blue). SNAP25 immunoreactivity was localized to the synaptic termini. View our protocol for [Fluorescent ICC Staining of Cells on Coverslips](#).

Immunocytochemistry



SNAP25 in PC-12 Rat Adrenal Pheochromocytoma Cells. PC-12 rat adrenal pheochromocytoma cells were cultured overnight in the presence of 50 ng/mL of Recombinant Rat b-NGF (Catalog # 556-NG) prior to immersion fixation. SNAP25 was detected using a Sheep Anti-Human/Mouse/Rat SNAP25 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF5946). The cells were stained with the NorthernLights 557-conjugated Donkey Anti-Sheep Affinity-purified IgG Secondary Antibody (red; Catalog # NL010). The cell nuclei were counterstained with DAPI (blue). SNAP25 immunoreactivity was localized to the synaptic termini. View our protocol for [Fluorescent ICC Staining of Cells on Coverslips](#).

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

SNAP25 (Synaptosomal associated protein of 25 kDa; also SUP) is a cytosolic 24-29 kDa member of the SNAP25 family of proteins. It is expressed in neurons, β -cells, and adrenal chromaffin cells. SNAP25 is involved in vesicle exocytosis, where two presynaptic SNAP25, and one syntaxin-1 molecule associates with vesicle membrane VAMP2 to form a α -helix via a zipper-like mechanism. This approximates two membranes, which subsequently fuse to create a pore. Human SNAP25b is 206 amino acids (aa) in length. It contains two t-SNARE coiled-coil homology domains (aa 19-81 and 140-202) and a proteolytic cleavage site at Arg180:Ile181. Palmitoylation occurs between aa 85-92; phosphorylation occurs on Thr138. There are three isoform variants. One shows nine aa changes over aa 58-89 (isoform a), a second shows a 30 aa substitution for aa 1-94, while a third contains an alternative start site at Met64. Full-length human and mouse SNAP25 are identical in amino acid sequence.