

ORDERING INFORMATION

Catalog Number: AF5485

Lot Number: CCIE01

Size: 100 µg

Formulation: 0.2 µm filtered solution in PBS with 5% trehalose

Storage: -20° C

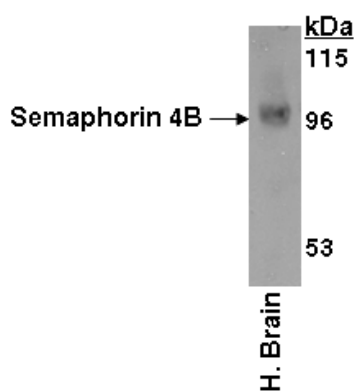
Reconstitution: sterile PBS

Specificity: human Semaphorin 4B extracellular domain

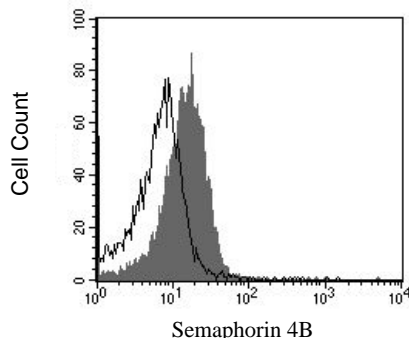
Immunogen: NS0-derived rhSemaphorin 4B isoform 1 extracellular domain

Ig Type: goat IgG

Applications: Western blot
Flow cytometry
Direct ELISA



Detection of Semaphorin 4B with AF5485. Tissue lysates were resolved by SDS-PAGE, transferred to an Immobilon-P membrane and immunoblotted with 1.0 µg/mL goat anti-hSemaphorin 4B.



THP-1 cells were stained with anti-Semaphorin 4B (R&D Systems, Cat. # AF5485, filled histogram), or control antibody (R&D Systems, Cat. # AB-108-C, open histogram) followed by PE-conjugated anti-goat IgG (R&D Systems Cat. # F0107).

Background

Semaphorin 4B (Sema4B; also SemaC) is a 95 - 100 kDa, class IV member of the Semaphorin family of proteins. It is expressed in neurons, and following PSD-95 induced clustering, participates in the formation or functioning of glutamatergic synapses. Mature human Sema4B is a type I transmembrane glycoprotein that is 794 amino acids (aa) in length. It contains a 674 aa extracellular region (aa 39 - 832) that is characterized by one Sema domain (aa 65 - 502), a PSI region (aa 520 - 574), and an Ig-like C2-type domain (aa 599 - 658). There is one potential soluble splice variant that shows a 29 aa substitution for aa 707 - 832. Over aa 39 - 712, human Sema4B shares 86% aa identity with mouse Sema4B.

Preparation

Produced in goats immunized with purified, NS0-derived, recombinant human Semaphorin 4B isoform 1 extracellular domain (rhSemaphorin 4B; aa 39 - 712; Accession # Q9NPR2). Human Semaphorin 4B specific IgG was purified by human Semaphorin 4B affinity chromatography.

Formulation

Lyophilized from a 0.2 µm filtered solution in phosphate-buffered saline (PBS) with 5% trehalose.

Reconstitution

Reconstitute with sterile PBS. If 0.5 mL of PBS is used, the antibody concentration will be 0.2 mg/mL.

Storage

Lyophilized samples are stable for twelve months from date of receipt when stored at -20° C to -70° C. Upon reconstitution, the antibody can be stored at 2° - 8° C for 1 month without detectable loss of activity. Reconstituted antibody can also be aliquotted and stored frozen at -20° C to -70° C in a manual defrost freezer for six months without detectable loss of activity. **Avoid repeated freeze-thaw cycles.**

Specificity

This antibody has been selected for its ability to recognize human Semaphorin 4B in the applications listed below. In direct ELISAs, this antibody shows approximately 15% cross-reactivity with rmSemaphorin 4B and less than 1% cross-reactivity with rhSemaphorin 3A, -4A and -7A.

Applications

Western blot - An antibody concentration of 1.0 µg/mL is recommended.

Flow cytometry - This antibody was tested for flow cytometry using THP-1 cells (Li, Z. *et al.* (2007) Anal. NY Acad. Sci **1106**:95). Dilute this antibody to 25 µg/mL and add 10 µL of the diluted solution to 1 - 2.5 x 10⁵ cells in a total reaction volume not exceeding 200 µL. The binding of unlabeled antibodies may be visualized by adding a secondary developing reagent such as anti-goat IgG conjugated to a fluorochrome. Between steps, wash cells in Flow Cytometry Staining Buffer (R&D Systems, Cat. # FC002) or equivalent.

Direct ELISA - This antibody can be used at 0.5 - 1.0 µg/mL with the appropriate secondary reagents to detect human Semaphorin 4B. The detection limit for rhSemaphorin 4B is approximately 0.5 ng/well.

Optimal dilutions should be determined by each laboratory for each application.