



Monoclonal Anti-human Semaphorin 3E Antibody

ORDERING INFORMATION

Catalog Number: MAB32391

Clone: 400513

Lot Number: ZJH01

Size: 100 µg

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

Storage: -20° C

Reconstitution: sterile PBS

Specificity: human Semaphorin 3E

Immunogen: NS0-derived rhSemaphorin 3E

Ig class: mouse IgG₁

Recommended Application:
Flow cytometry

Other application:
Direct ELISA

Background

Semaphorin 3E (previously SemaH) is a secreted protein belonging to the semaphorin family. Semaphorin 3E is highly expressed by a subset of motor neurons in developing somites, where it acts as a repulsive cue for PlexinD1-expressing endothelial cells of adjacent intersomitic vessels. Like other Semaphorin 3E proteins, the 775 aa human Semaphorin 3E contains a signal sequence, a sema domain, a C2-like Ig domain and a C-terminal basic domain. Disulfide-linked dimerization and furin-type proteolytic processing increase Semaphorin 3E bioactivity. Mature human and mouse Semaphorin 3E share 90% aa identity.

Preparation

This antibody was produced from a hybridoma resulting from the fusion of a mouse myeloma with B cells obtained from a mouse immunized with purified, NS0-derived, recombinant human Semaphorin 3E (rhSemaphorin 3E; aa 25 - 775; Accession #O15041). The IgG fraction of the tissue culture supernatant was purified by Protein G 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 0.2 mL of PBS is used, the antibody concentration will be 500 µg/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

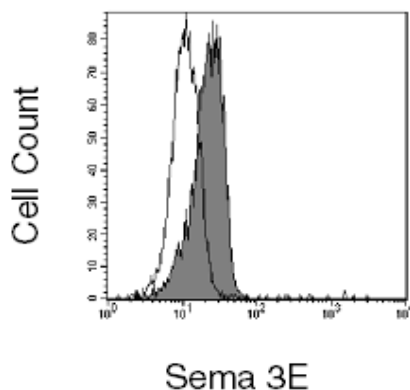
This antibody detects human Semaphorin 3E in direct ELISAs. In this application, this antibody shows 15% cross-reactivity with rhSemaphorin 3B and no cross-reactivity with rhSemaphorin 6A.

Applications

Flow cytometry - This antibody was validated for flow cytometry using PHA-activated T cells. For intracellular staining to detect Semaphorin 3E, cells must first be fixed and permeabilized using 4% paraformaldehyde and 0.1% saponin in PBS. Dilute this antibody to 25 µg/mL and add 10 µL of the diluted solution to 1 - 5 x 10⁵ cells in a total reaction volume not exceeding 200 µL. The binding of unlabeled monoclonal antibodies may be visualized by adding a secondary developing reagent such as goat anti-mouse IgG conjugated to a fluorochrome.

Direct ELISA - This antibody can be used at 0.5 - 1.0 µg/mL with the appropriate secondary reagents to detect human Semaphorin 3E. The detection limit for rhSemaphorin 3E is approximately 6 ng/well.

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



PHA-activated T cells were stained with anti-Sema 3E (R&D Systems Cat. # MAB32391, filled histogram) or isotype control antibody (R&D Systems, Cat. # MAB002, open histogram), followed by PE-conjugated anti-mouse antibody (R&D Systems, Cat. # F0102B).

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