Coud-Clone Corp.

APL930Hu01 100µg Active Semaphorin 3F (SEMA3F) Organism Species: *Homo sapiens (Human) Instruction manual*

FOR RESEARCH USE ONLY NOT FOR USE IN CLINICAL DIAGNOSTIC PROCEDURES

1st Edition (Apr, 2016)

[PROPERTIES]

Source: Prokaryotic expression. Host: *E. coli* Residues: Val350~Leu483 Tags: N-terminal His-tag Purity: >95% Endotoxin Level: <1.0EU per 1µg (determined by the LAL method). Buffer Formulation: 20mM Tris, 150mM NaCl, pH8.0, containing 0.05% sarcosyl and 5% trehalose. Applications: Cell culture; Activity Assays. (May be suitable for use in other assays to be determined by the end user.) Predicted isoelectric point: 9.2 Predicted Molecular Mass: 16.6kDa Accurate Molecular Mass: 16kDa as determined by SDS-PAGE reducing conditions.

[<u>USAGE</u>]

Reconstitute in 20mM Tris, 150mM NaCl (pH8.0) to a concentration of 0.1-1.0 mg/mL. Do not vortex.

[STORAGE AND STABILITY]

Storage: Avoid repeated freeze/thaw cycles.

Store at 2-8°C for one month.

Aliquot and store at -80°C for 12 months.

Cloud-Clone Corp.

Stability Test: The thermal stability is described by the loss rate. The loss rate was determined by accelerated thermal degradation test, that is, incubate the protein at 37°C for 48h, and no obvious degradation and precipitation were observed. The loss rate is less than 5% within the expiration date under appropriate storage condition.

[<u>SEQUENCE</u>]

RNPVIYAVFT SSGSVFRGSA VCVYSMADIR MVFNGPFAHK EGPNYQWMPF SGKMPYPRPG TCPGGTFTPS MKSTKDYPDE VINFMRSHPL MYQAVYPLQR RPLVVRTGAP YRLTTIAVDQ VDAADGRYEV LFL

V

[ACTIVITY]

Semaphorin-3F (SEMA3F) is a member of semaphorins family. All the family members have a secretion signal, a 500-amino acid sema domain, and 16 conserved cysteine residues. Semaphorins not only guide axons in development, but also have major roles in immune function (classes 4, 6, and 7) and the development of bones. Class 3 semaphorins are one of the most versatile semaphorin classes, in which Sema3a is the most studied. Besides, SEMA3F is deleted or downregulated in many metastatic tumors. To test the effect of SEMA3F on inhibit cell proliferation, human lung carcinoma cells A549 were seeded into triplicate wells of 96-well plates at a density of 5,000 cells/well and allowed to attach, replaced with serum-free overnight, then the medium was replaced with 2% serum standard DMEM prior to the addition of various concentrations of recombinant human SEMA3F. After incubated for 48h, cells were observed by inverted microscope and cell proliferation was measured by Cell Counting Kit-8 (CCK-8). Briefly, 10µL of CCK-8 solution was added to each well of the plate, then the absorbance at 450nm was measured using a microplate reader after incubating the plate for 1-4 hours at 37°C. Proliferation of A549 cells after incubation with SEMA3F for 48h observed by inverted microscope was shown in

Cloud-Clone Corp.

Figure 1. Cell viability was assessed by CCK-8 assay after incubation with recombinant SEMA3F for 48h. The result was shown in Figure 2. It was obvious that SEMA3F significantly increased cell viability of A549 cells.



Figure 1. Cell proliferation of A549 cells inhibiton by stimulating with SEMA3F.

(A) A549 cells cultured in DMEM, stimulated with 100ng/mL SEMA3F for 48h;

(B) Unstimulated A549 cells cultured in DMEM for 48h.



Recombinant Human SEMA3F (ng/ml)



[IDENTIFICATION]



Figure 3. SDS-PAGE

Sample: Active recombinant SEMA3F, Human



Figure 4. Western Blot

Sample: Recombinant SEMA3F, Human;

Antibody: Rabbit Anti-Human SEMA3F Ab (PAL930Hu01)

[IMPORTANT NOTE]

The kit is designed for in vitro and research use only, we will not be responsible for any issue if the kit was used in clinical diagnostic or any other procedures.