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APL917Ra01 100µg Active Semaphorin 3A (SEMA3A) Organism Species: *Rattus norvegicus (Rat) Instruction manual* 

#### FOR RESEARCH USE ONLY NOT FOR USE IN CLINICAL DIAGNOSTIC PROCEDURES

13th Edition (Revised in Aug, 2023)

#### [PROPERTIES]

Source: Prokaryotic expression. Host: *E. coli* Residues: Arg31~Pro150 Tags: N-terminal His-tag Purity: >90% Endotoxin Level: <1.0EU per 1µg (determined by the LAL method). Buffer Formulation: PBS, pH7.4, containing 0.01% SKL, 5%Trehalose . Original Concentration: 200µg/mL Applications: Cell culture; Activity Assays. (May be suitable for use in other assays to be determined by the end user.) Predicted isoelectric point: 8.9 Predicted Molecular Mass: 17.6kDa Accurate Molecular Mass: 17kDa as determined by SDS-PAGE reducing conditions.

### [ <u>USAGE</u> ]

Reconstitute in 10mM PBS (pH7.4) 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.

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**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.

#### [SEQUENCE]

RLKLSYKEML ESNNVITFNG LANSSSYHTF LLDEERSRLY VGAKDHIFSF NLVNIKDFQK IVWPVSYTRR DECKWAGKDI LKECANFIKV LKAYNQTHLY ACGTGAFHPI CTYIEVGHHP

#### [ACTIVITY]

The Semaphorin 3A(SEMA3A) which belongs to the semaphorin family can function as either a chemorepulsive agent, inhibiting axonal outgrowth, or as a chemoattractive agent, stimulating the growth of apical dendrites. In both cases, the protein is vital for normal neuronal pattern development. Semaphorin 3A is secreted protein containing a Sema domain, an immunoglobulin C2-like domain and a basic domain near the carboxyl tail. It can be secreted by neurons and surrounding tissue to guide migrating cells and axons in the developing nervous system. Besides, Neuropilin 1 (NRP1) has been identified as an interactor of SEMA3A, thus a functional binding ELISA assay was conducted to detect the interaction of recombinant rat SEMA3A and recombinant human NRP1. Briefly, SEMA3A were diluted serially in PBS, with 0.01% BSA (pH 7.4). Duplicate samples of 100 ul were then transferred to NRP1-coated microtiter wells and incubated for 2h at 37 °C. Wells were washed with PBST and incubated for 1h with anti-SEMA3A pAb, then aspirated and washed 3 times. After incubation with HRP labelled secondary antibody, wells were aspirated and washed 3 times. With the addition of substrate solution , wells were incubated 15-25 minutes at  $37^{\circ}$ C. Finally, add 50 ul stop solution to the wells and read at 450 nm immediately. The binding

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activity of recombinant rat SEMA3A and recombinant human NRP1 was shown in Figure 1, the EC50 for this effect is 1.22 ug/mL.





### [IDENTIFICATION]



Figure 2. Gene Sequencing (extract)

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kDa 70
44
33
26
22
18
14
 10

Figure 3. SDS-PAGE

Sample: Active recombinant SEMA3A, Rat

#### [IMPORTANT NOTE]

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