APB310Ra01 100µg Active Elastase 4 (ELA4) Organism Species: Rattus norvegicus (Rat) *Instruction manual*

FOR IN VITRO USE AND RESEARCH USE ONLY NOT FOR USE IN CLINICAL DIAGNOSTIC PROCEDURES

1st Edition (Apr, 2016)

[PROPERTIES]

Source: Prokaryotic expression.

Host: E. coli

Residues: Val30~leu268

Tags: N-terminal His-tag

Purity: >95%

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: 5.9

Predicted Molecular Mass: 30.2kDa

Accurate Molecular Mass: 30kDa 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.

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>]

V VGGEDAVPNS WPWQVSLQYL KDDTWRHTCG GSLITTSHVL TAAHCINKDF TYRVGLGKYN LTVEDEEGSV YAEVDTIYVH EKWNRLFLWN DIAIIKLAEP VELSNTIQVA CIPEEGSLLP QDYPCYVTGW GRLWTNGPIA EVLQQGLQPI VSHATCSRLD WWFIKVRKTM VCAGGDGVIS ACNGDSGGPL NCQAEDGSWQ VHGIVSFGSS SGCNVHKKPV VFTRVSAYND WINEKIQL

[ACTIVITY]

Elastase 4 (ELA4) is a member of the peptidase S1 family. The encoded protein is a serum calcium-decreasing factor that has chymotrypsin-like protease activity. It regulates activation and degradation of trypsinogens and procarboxypeptidases by targeting specific cleavage sites within their zymogen precursors. Has chymotrypsin-type protease activity and hypocalcemic activity. Besides, Plasminogen Activator, Urokinase Receptor (uPAR) has been identified as an interactor of ELA4, thus a binding ELISA assay was conducted to detect the interaction of recombinant rat ELA4 and recombinant rat uPAR. Briefly, ELA4 were diluted serially in PBS, with 0.01%BSA (pH 7.4). Duplicate samples of 100uL were then transferred to uPAR-coated microtiter wells and incubated for 2h at 37 °C. Wells were washed with PBST and incubated for 1h with anti-ELA4 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°C. Finally, add 50µL stop solution to the wells and read at 450nm immediately. The binding activity of of ELA4 and uPAR was shown in Figure 1, and this effect was in a dose dependent manner.

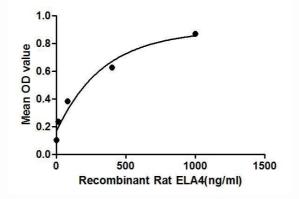


Figure 1. The binding activity of ELA4 with uPAR.

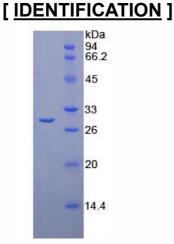
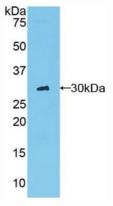


Figure 2. SDS-PAGE

Sample: Active recombinant ELA4, Rat





Sample: Recombinant ELA4, Rat;

Antibody: Rabbit Anti-Rat ELA4 Ab (PAB310Ra01)