APA181Mu61 50µg Active Neutrophil Elastase (NE) Organism Species: *Mus musculus (Mouse) Instruction manual* 

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

12th Edition (Revised in Aug, 2016)

#### [PROPERTIES]

Source: Eukaryotic expression.

Host: 293F cell

Residues: Ser27~Asn265

Tags: N-terminal His-tag

**Purity: >97%** 

Endotoxin Level: <1.0EU per 1µg (determined by the LAL method).

Buffer Formulation: PBS, pH7.4, containing 5% Trehalose .

Original Concentration: 500µg/mL

Applications: Cell culture; Activity Assays.

(May be suitable for use in other assays to be determined by the end user.)

Predicted isoelectric point: 9.6

Predicted Molecular Mass: 21.7kDa

**Accurate Molecular Mass:** 33kDa as determined by SDS-PAGE reducing conditions. Phenomenon explanation:

The possible reasons that the actual band size differs from the predicted are as follows:

1. Splice variants: Alternative splicing may create different sized proteins from the same gene.

2. Relative charge: The composition of amino acids may affects the charge of the protein.

3. Post-translational modification: Phosphorylation, glycosylation, methylation etc.

4. Post-translation cleavage: Many proteins are synthesized as pro-proteins, and then cleaved to give the active form.

5. Polymerization of the target protein: Dimerization, multimerization etc.

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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.

**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]

SEIVGGRPARPHAWPFMASLQRRGGHFCGATLIARNFVMSAAHCVNGLNFRSVQVVLGAHDLRRQERTRQTFSVQRIFENGFDPSQLLND IVIIQLNGSATINANVQVAQLPAQGQGVGDRTPCLAMGWGRLGTNRPSPSVLQELNVTVVTNMCRRRVNVCTLVPRRQAGICFGDSGGPL VCNNLVQGIDSFIRGGCGSGLYPDAFAPVAEFADWINSIIRSHNDHLLTHPKDREGRTN

# [ACTIVITY]

Neutrophil elastase (NE), also known as polymorphonuclear leukocyte elastase, is a major protease in the primary granules of neutrophils, is involved in microbicidal activity. It is located primarily in the azurophil granules of polymorphonuclear leukocytes. NE is an important factor promoting inflammation, has bactericidal effects, and shortens the inflammatory process. NE also regulates tumor growth by promoting metastasis and tumor microenvironment remodeling. However, NE plays a role in killing tumors under certain conditions and promotes other diseases such as pulmonary ventilation dysfunction. Additionally, it plays a complex role in various physiological processes and mediates several diseases. Lactoferrin (LTF) has been identified as an interactor of NE, thus a functional binding ELISA assay was conducted to detect the interaction of recombinant mouse NE and recombinant goat LTF. Briefly, biotin-linked NE were diluted serially in PBS, with 0.01% BSA (pH 7.4). Duplicate samples of 100  $\mu$  I were then transferred to LTF-coated microtiter wells and incubated for 1h at 37°C.

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Wells were washed with PBST 3 times and incubation with Streptavidin-HRP for 30min, then wells were aspirated and washed 5 times. With the addition of substrate solution, wells were incubated 15-25 minutes at 37  $^{\circ}$ C. Finally, add 50 µl stop solution to the wells and read at 450 nm immediately. The binding activity of NE and LTF was shown in Figure 1, the EC50 for this effect is 0.084 ug/mL.

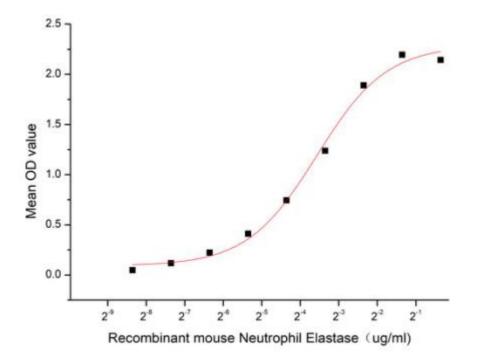


Figure 1. The binding activity of recombinant mouse NE and recombinant goat LTF

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### [IDENTIFICATION]

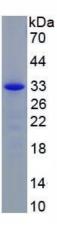


Figure 2. SDS-PAGE Sample: Active recombinant NE, Mouse

# [<u>IMPORTANT NOTE</u>]

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.