APB785Hu61 100μg
Active Neprilysin (CD10)
Organism Species: *Homo sapiens* (Human)
Instruction manual

FOR RESEARCH USE ONLY
NOT FOR USE IN CLINICAL DIAGNOSTIC PROCEDURES

13th Edition (Revised in Aug, 2023)

[PROPERTIES]

Source: Eukaryotic expression.
Host: 293F cell
Residues: Tyr52~Trp750
Tags: N-terminal His-tag
Purity: >95%
Endotoxin Level: <1.0EU per 1μg (determined by the LAL method).
Buffer Formulation: PBS, pH7.4, containing 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: 5.2
Predicted Molecular Mass: 81.6kDa
Accurate Molecular Mass: 100kDa 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.

[USAGE]

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]

YDDGICKSS DCIKSAARLI QNMDATTEPC TDFFKYACGG WLKRNVIPET
SSRYGNFDIL RDELEVVLKD VLQEPKTEDI VAVQKAKALY RSCINESAID
SRGGEPLLKL LPDIYGWPVA TENWEQKYGA SWTAEKAIAQ LNSKYGKKVL
INLFVGTDDK NSVNHVIHID QPRLGLPSRD YYECTGIYKE ACTAYVDFMI
SVARLIRQEE RLPIDENQLA LEMNKVMELE KEIANATAKP EDRNDPMLLY
NKMTLAQIQN NFSLEINGKP FSWLNFTNEI MSTVNISITN EEDVVVYAPE
YLTKLKPILT KYSARDLQNL MSWRFIMDLV SSLSRTYKES RNAFRKALYG
TTSETATWRR CANYVNGNME NAVGRLYVEA AFAGESKHVV EDLIAQIREV
FIQTLDDLTW MDAETKKRAE EKALAIKERI GYPDDIVSND NKLNNEYLEL
NYKEDEYFEN IIQNLKFSQS KQLKKLREKV DKDEWISGAA VVNAFYSSGR
NQIVFPAGIL QPPFFSAQQS NSLNYGGIGM VIGHEITHGF DDNGRNFNKD
GDLVDWWTQQ SASNFKEQSQ CMVYQYGNFS WDLAGGQHLN GINTLGENIA
DNGGLGQAYR AYQNYIKKNG EEKLLPGLDL NHKQLFFLNF AQVWCGTYRP
EYAVNSIKTD VHSPGNFRII GTLQNSAEFS EAFHCRKNSY MNPEKKCRVW

[ACTIVITY]

Neprilysin/CD10, also known as NEP and neutral endopeptidase, is a zinc metallopeptidase expressed at the cell surface of a variety of cells. The enzyme functions both as an endopeptidase with a thermolysin-like specificity and as a dipeptidylcarboxypeptidase. NEP has been shown to be involved in the

degradation of enkephalins in the mammalian brain and the inactivation of circulating atrial natriuretic peptide. NEP has also been identified as the common acute lymphocytic leukemia antigen (CALLA), and is expressed on the surface of lymphocytes in some disease states. The activity of recombinant human NEP is measured by its ability to cleave a fluorogenic peptide substrate MCA-Arg-Pro-Pro-Gly-Phe-Ser-Ala-Phe-Lys(DNP)-OH in the assay buffer 50 mM Tris, 0.05% Brij-35, pH 9.0. The rhNEP is diluted to 0.1 ug/ml in assay buffer, then add 50 µL protein into a black well plate and start the reaction by adding 50 µL of 20 µM substrate (diluted by assay buffer), with a substrate blank containing 50 µL assay buffer, 50 µL substrate, and no rhNEP. Then read at excitiation and emission wavelengths of 320 nm and 405 nm, respectively, in kinetic mode for 5 minutes. The specific activity of recombinant human NEP is > 3500 pmol/min/µg.

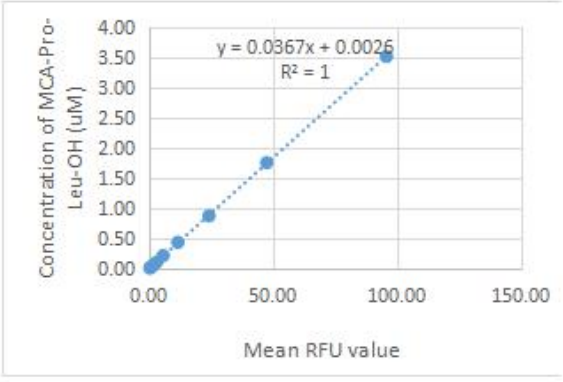

Figure 1. The standard curve of MCA-Pro-Leu-OH

RFU (320/405)	MCA-Pro-Leu-OH (product) uM
95.78	3.52
47.46	1.76
24.20	0.88
11.63	0.44
5.71	0.22
3.05	0.11
1.52	0.05
0.77	0.03

Specific Activity (pmol/min/µg) =

$$\frac{\text{Adjusted Vmax}*\text{(RFU/min)} \times \text{Conversion Factor}**\text{(pmol/RFU)}}{\text{amount of enzyme (ug)}}$$

*Adjusted for Substrate Blank

**Derived using calibration standard MCA-Pro-Leu-OH

[IDENTIFICATION]

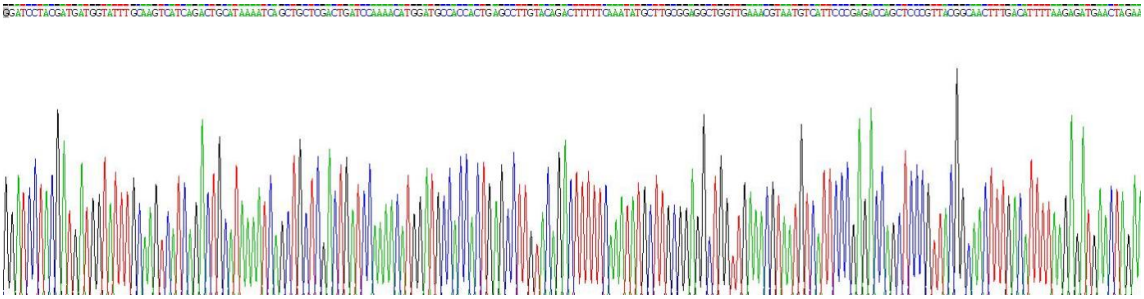

Figure 2. Gene Sequencing (extract)

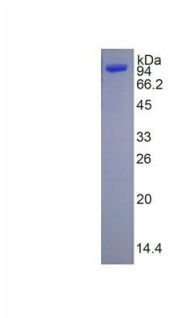

Figure 3. SDS-PAGE

Sample: Active recombinant CD10, Human

[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.