

APB280Hu62 100μg Active Cathepsin D (CTSD)

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: Leu21~Leu412 Tags: N-terminal His-tag

**Purity: >90%** 

**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: 6.5

Predicted Molecular Mass: 44.2kDa

Accurate Molecular Mass: 44kDa as determined by SDS-PAGE reducing conditions.

#### [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

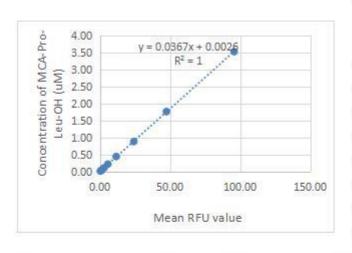
protein at 37°C for 48h, and no obvious degradation and precipitation were observed. The loss rate is less than 5% within the expiration date under appropriate storage condition.

## [SEQUENCE]

LVRIPLHKFTSIRRTMSEVGGSVEDLIAKGPVSKYSQAVPAVTEGPIPEVLKNYMDAQYYGEIGIGTPPQCFTVVFDTGSSNLWVPSI HCKLLDIACWIHHKYNSDKSSTYVKNGTSFDIHYGSGSLSGYLSQDTVSVPCQSASSASALGGVKVERQVFGEATKQPGITFIAAKFD GILGMAYPRISVNNVLPVFDNLMQQKLVDQNIFSFYLSRDPDAQPGGELMLGGTDSKYYKGSLSYLNVTRKAYWQVHLDQVEVASGLT LCKEGCEAIVDTGTSLMVGPVDEVRELQKAIGAVPLIQGEYMIPCEKVSTLPAITLKLGGKGYKLSPEDYTLKVSQAGKTLCLSGFMG MDIPPPSGPLWILGDVFIGRYYTVFDRDNNRVGFAEAARL

#### [ACTIVITY]

Cathepsin D is a lysosomal aspartic protease of the pepsin family. Human cathepsin D is synthesized as a precursor protein, consisting of a signal peptide (residues 1-18), a propeptide (residues 19-64), and a mature chain (residues 65-412). The mature chain can be processed further to the light (residues 65-161) and heavy (residues 169-412) chains. It is expressed in most cells and overexpressed in breast cancer cells. It is a major enzyme in protein degradation in lysosomes, and also involved in the presentation of antigenic peptides. Mice deficient in this enzyme showed a progressive atrophy of the intestinal mucosa, a massive destruction of lymphoid organs, and a profound neuronal ceroid lipofucinosis, indicating that cathepsin D is essential for proteolysis of proteins regulating cell growth and tissue homeostasis. The activity of recombinant human CTSD is measured by its ability to cleave a fluorogenic peptide substrate MCA-Pro-Leu-Gly-Leu-DPA-Ala-Arg-NH2 in the assay buffer 0.1 M NaOAc, 0.2 M NaCl, pH 3.5. The rhCTSD is diluted to 20 ug/ml in assay buffer, then incubated at 37 ° C for 30min. The incubated rhCTSD is diluted to 5 ug/mL in assay buffer. Loading into a black well plate 50 µL of 5 µg/mL rhCTSD and start the reaction by adding 50 µL of 60 µM substrate, with a substrate blank containing 50 µL assay buffer, 50 µL substrate, and no rhCTSD. 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 CTSD is > 675 pmol/min/µg.



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

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

Specific Activity (pmol/min/µg) =

Adjusted Vmax \*(RFU/min) x Conversion Factor \*\*(pmol/RFU)
amount of enzyme (ug)

# [ IDENTIFICATION ]

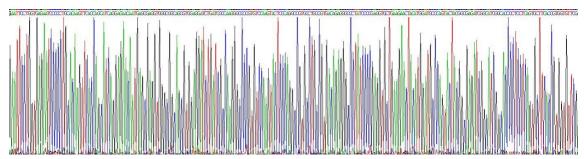


Figure 2. Gene Sequencing (extract)

<sup>\*</sup>Adjusted for Substrate Blank

<sup>\*\*</sup>Derived using calibration standard MCA-Pro-Leu-OH

# Cloud-Clone Corp.

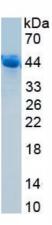


Figure 3. SDS-PAGE

Sample: Active recombinant CTSD, 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.