APB280Hu61 100μg
Active Cathepsin D (CTSD)
Organism Species: *Homo sapiens* (Human)
Instruction manual

FOR RESEARCH USE ONLY
NOT FOR USE IN CLINICAL DIAGNOSTIC PROCEDURES

1st Edition (Apr, 2016)

[PROPERTIES]

Source: Eukaryotic expression.
Host: 293F cell
Residues: Leu21~Leu412
Tags: N-terminal His-tag
Purity: >98%
Endotoxin Level: <1.0EU per 1μg (determined by the LAL method).
Buffer Formulation: 10mM PBS, pH7.6, containing 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: 6.1
Predicted Molecular Mass: 44.2kDa
Accurate Molecular Mass: 50kDa 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 (pH7.6) 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]

LVRIPLHKFT SIRRTMSEVG GSVEDLIAKG
PVSKYSQAVP AVTEGPIPEV LKNYMDAQYY GEIGIGTPPQ CFTVVFDTGS
SNLWVPSIHC KLLDIACWIH HKYNSDKSST YVKNGTSFDI HYGSGSLSGY
LSQDTVSVPC QSASSASALG GVKVERQVFG EATKQPGITF IAAKFDGILG
MAYPRISVNN VLPVFDNLMQ QKLVDQNIFS FYLSRDPDAQ PGGELMLGGT
DSKYYKGSLS YLNVTRKAYW QVHLDQVEVA SGLTLCKEGC EAIVDTGTSL
MVGPVDEVRE LQKAIGAVPL IQGEYMIPCE KVSTLPAITL KLGGKGYKLS
PEDYTLKVSQ AGKTLCLSGF MGMDIPPPSG PLWILGDVFI GRYYTVFDRD
NNRVGFAEAA RL

[ACTIVITY]

Cathepsin D (CSTD) is an aspartic protease that depends critically on protonation of its active site Asp residue. Along with Asp-protonation, lower pH also leads to conformational switch in cathepsin-D: the N-terminal segment of the protease moves out of the active site as pH drops. Similar to other aspartic protainases, cathepsin D accommodates up to 8 amino acid residues in the binding cleft of the active site. The main physiological functions of cathepsin D consist of metabolic

degradation of intracellular proteins, activation and degradation of polypeptide hormones and growth factors, activation of enzymatic precursors, processing of enzyme activators and inhibitors, brain antigen processing and regulation of programmed cell death. Besides, Heat Shock Protein 90kDa Beta 1 (HSP90b1) has been identified as an interactor of CSTD, thus a binding ELISA assay was conducted to detect the interaction of recombinant human CSTD and recombinant human HSP90b1. Briefly, CSTD were diluted serially in PBS, with 0.01% BSA (pH 7.4). Duplicate samples of 100μL were then transferred to HSP90b1-coated microtiter wells and incubated for 2h at 37℃. Wells were washed with PBST and incubated for 1h with anti-CSTD 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℃. Finally, add 50μL stop solution to the wells and read at 450nm immediately. The binding activity of CSTD and HSP90b1 was shown in Figure 1, and this effect was in a dose dependent manner.

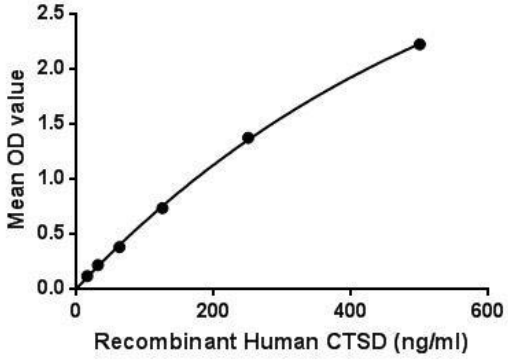

Figure 1. The binding activity of CTSD with HSP90b1.

[IDENTIFICATION]

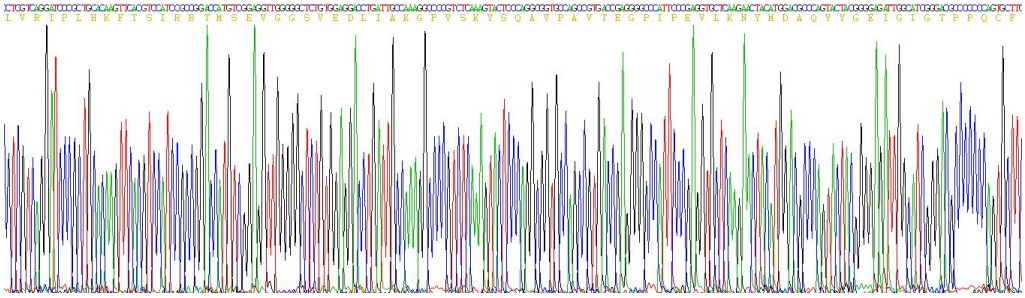

Figure 1. Gene Sequencing (extract)

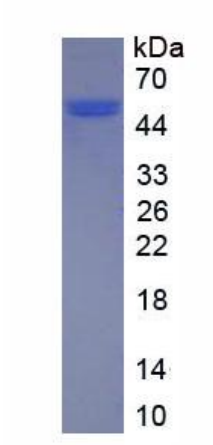

Figure 2. SDS-PAGE

Sample: Active recombinant CTSD, Human

[IMPORTANT NOTE]

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