

**APC960Hu01 10µg**

**Active Calpain 3 (CAPN3)**

**Organism Species: *Homo sapiens* (Human)**

***Instruction manual***

FOR RESEARCH USE ONLY

NOT FOR USE IN CLINICAL DIAGNOSTIC PROCEDURES

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1st Edition (Apr, 2016)

## **[ PROPERTIES ]**

**Source:** Prokaryotic expression.

**Host:** *E. coli*

**Residues:** Ile602~Ala821

**Tags:** N-terminal His-tag

**Purity:** >95%

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

**Buffer Formulation:** 100mM NaHCO<sub>3</sub>, 500mM NaCl, pH8.3, containing 0.01% sarcosyl, 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:** 5.5

**Predicted Molecular Mass:** 29.1kDa

**Accurate Molecular Mass:** 32kDa as determined by SDS-PAGE reducing conditions.

## **[ USAGE ]**

Reconstitute in 100mM NaHCO<sub>3</sub>, 500mM NaCl (pH8.3) 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 ]**

```
IIFVSDRAN SNKELGVDQE SEEGKGTSP DKQKQSPQPQ PGSSDQESEE  
QQQFRNIFKQ IAGDDMEICA DELKKVLNTV VNKHKDLKTH GFTLESCSRM  
IALMDTDGSG KLNQLQEFHHL WNKIKAWQKI FKHYDTDQSG TINSYEMRNA  
VNDAGFHLNN QLYDIITMRY ADKHMNIDFD SFICCFVRLE GMFRAFHAFD  
KDG DGI I KLN VLEWLQ L TMY A
```

## **[ ACTIVITY ]**

Calpain 3 is a calcium-dependent cysteine protease mainly expressed in skeletal muscle. In humans, calpain 3 is encoded by the CAPN3 gene. This gene encodes a muscle-specific member of the calpain large subunit family that specifically binds to titin. Mutations in this gene are associated with limb-girdle muscular dystrophies type 2A. Alternate promoters and alternative splicing result in multiple transcript variants encoding different isoforms and some variants are ubiquitously expressed. Besides, Titin (TTN) has been identified as an interactor of CAPN3, thus a binding ELISA assay was conducted to detect the interaction of recombinant human CAPN3 and recombinant human TTN. Briefly, CAPN3 were diluted serially in PBS with 0.01% BSA (pH 7.4). Duplicate samples of 100uL were then transferred to TTN-coated microtiter wells and incubated for 2h at 37°C. Wells were washed with PBST and incubated for 1h with anti-CAPN3 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 CAPN3 and TTN was shown in Figure 1, and this effect was in a dose dependent manner.

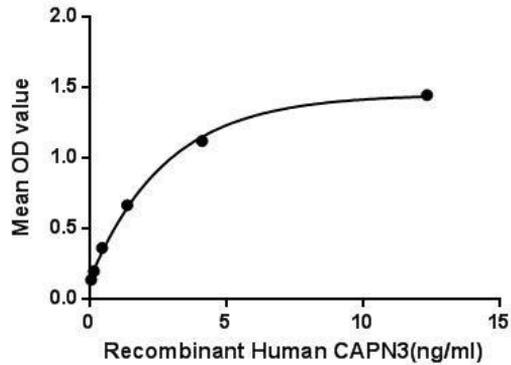


Figure 1. The binding activity of CAPN3 with TTN.

## [ IDENTIFICATION ]

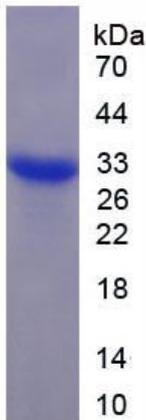
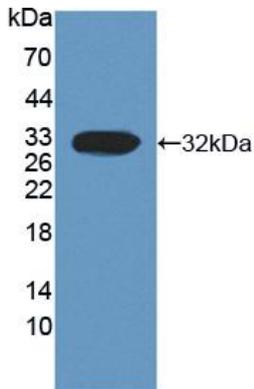


Figure 2. SDS-PAGE

Sample: Active recombinant CAPN3, Human



**Figure 3. Western Blot**

**Sample: Recombinant CAPN3, Human;**

**Antibody: Rabbit Anti-Human CAPN3 Ab (PAC960Hu01)**

**[ 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.