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APA478Hu61 100µg Active Cardiac Troponin I (cTnl) 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: Ala2~Ser210 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: Activity Assays. (May be suitable for use in other assays to be determined by the end user.) Predicted isoelectric point: 10.6 Predicted Molecular Mass: 25.5kDa 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. [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]

ADGSSDAAR EPRPAPAPIR RRSSNYRAYA TEPHAKKKSK ISASRKLQLK TLLLQIAKQE LEREAEERRG EKGRALSTRC QPLELAGLGF AELQDLCRQL HARVDKVDEE RYDIEAKVTK NITEIADLTQ KIFDLRGKFK RPTLRRVRIS ADAMMQALLG ARAKESLDLR AHLKQVKKED TEKENREVGD WRKNIDALSG MEGRKKKFES

# [ACTIVITY]

Cardiac Troponin I (cTnI), as a cytoplasmic protein, is one of the most important regulatory proteins part of the troponin-tropomyosin complex localized on the actin (thin) myofilaments in cardiac myocytes. It is the inhibitory subunit of troponin, the thin filament regulatory complex which confers calcium-sensitivity to striated muscle actomyosin ATPase activity. Besides, Actin Alpha 1, Cardiac Muscle (ACTC1) has been identified as an interactor of cTnI, thus a functional binding ELISA assay was conducted to detect the interaction of recombinant human cTnI and recombinant human ACTC1.

Briefly, biotin-linked cTnI were diluted serially in PBS, with 0.01% BSA (pH 7.4). Duplicate samples of 100  $\mu$  I were then transferred to ACTC1-coated microtiter wells and incubated for 1h at 37 °C. 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

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minutes at 37  $^\circ\!C$ . Finally, add 50µl stop solution to the wells and read at 450nm immediately. The binding activity of cTnl and ACTC1 was shown in Figure 1, the EC50 for this effect is 0.066ug/mL.

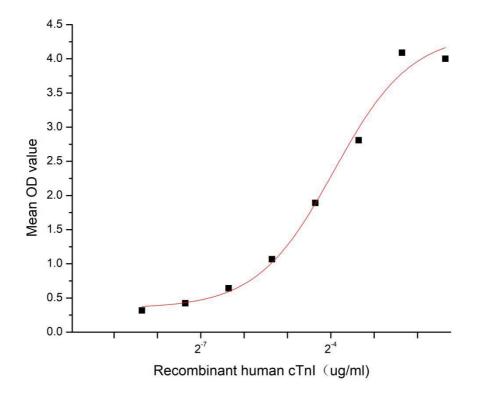
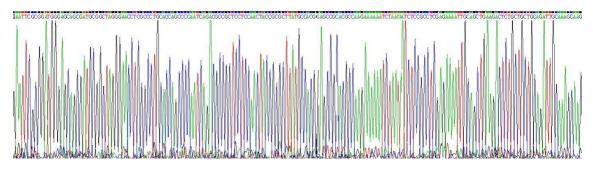


Figure 1. The binding activity of recombinant human cTnl and recombinant human ACTC1

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# [ IDENTIFICATION ]



#### Figure 2. Gene Sequencing (extract)

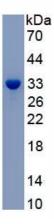


Figure 3. SDS-PAGE

Sample: Active recombinant cTnl, 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.