

APA478Hu01 100µg
Active Active Troponin I Type 3, Cardiac (TNNI3)
Organism Species: *Homo sapiens* (Human)
Instruction manual

FOR RESEARCH USE ONLY
NOT FOR USE IN CLINICAL DIAGNOSTIC PROCEDURES

1st Edition (Apr, 2016)

[PROPERTIES]

Source: Prokaryotic expression.

Host: *E. coli*

Residues: Met1~Gly203

Tags: N-terminal His-tag

Purity: >98%

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

Buffer Formulation: 20mM Tris, 150mM NaCl, pH8.0, containing 0.05% sarcosyl and 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: 9.7

Predicted Molecular Mass: 24.4kDa

Accurate Molecular Mass: 28kDa 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 20mM Tris, 150mM NaCl (pH8.0) 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]

MADGSSDAAR EPRPAPAPIR RRSSNYRAYA TEPHAKKSK ISASRKLQLK
TLLLQIAKQE LEREAERRG EKGRALSTRC QPLELAGLGF AELQDLCRQL
HARVDKVDEE RYDIEAKVTK NITEIADLTQ KIFDLRGKFK RPTLRRVRIS
ADAMMQALLG ARAKESDLDR AHLKQVKED TEKENREVGD WRKNIDALSG
MEG

[ACTIVITY]

Troponin I Type 3, Cardiac (TNNI3) is a protein that in humans is encoded by the TNNI3 gene. It is a tissue-specific subtype of troponin I, which in turn is a part of the troponin complex. The TNNI3 gene encoding cardiac troponin I (cTnI) is located at 19q13.4 in the human chromosomal genome. Human cTnI is a 24kDa protein consisting of 210 amino acids with isoelectric point (pI) of 9.87. cTnI is exclusively expressed in adult cardiac muscle. Besides, Troponin T Type 2, Cardiac (TNNT2) has been identified as an interactor of TNNI3, thus a binding ELISA assay was conducted to detect the interaction of recombinant human TNNI3 and recombinant human TNNT2. Briefly, TNNI3 was diluted serially in PBS

with 0.01% BSA (pH 7.4). Duplicate samples of 100 μ L were then transferred to TNNT2-coated microtiter wells and incubated for 2h at 37 $^{\circ}$ C. Wells were washed with PBST and incubated for 1h with anti-TNNI3 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 $^{\circ}$ C. Finally, add 50 μ L stop solution to the wells and read at 450nm immediately. The binding activity of TNNI3 and TNNT2 was shown in Figure 1, and this effect was in a dose dependent manner.

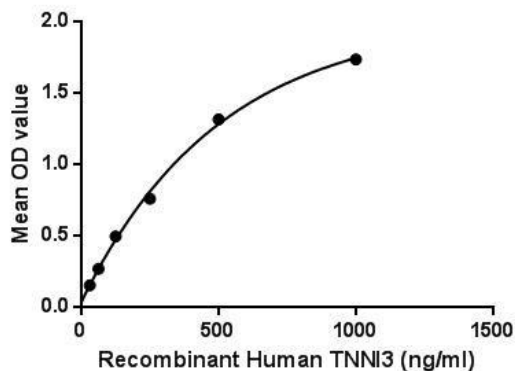


Figure 1. The binding activity of TNNI3 with TNNT2.

[IDENTIFICATION]

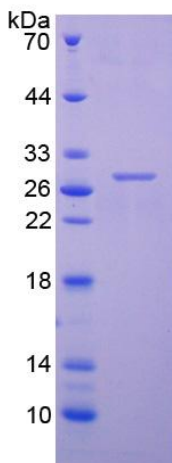


Figure 2. SDS-PAGE

Sample: Active recombinant TNNI3, Human

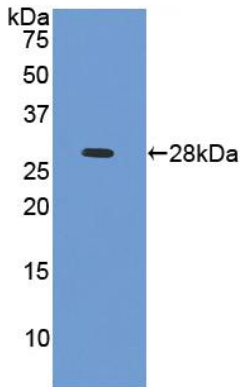


Figure 3. Western Blot

Sample: Recombinant TNNI3, Human;

Antibody: Rabbit Anti-Human TNNI3 Ab (PAA478Hu01)

[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.