APD229Hu01 100µg Active Troponin I Type 1, Slow Skeletal (TNNI1) 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: Prokaryotic expression. Host: E. coli **Residues:** Met1~Asp103 linked with Leu142~Gln187 Tags: N-terminal His-tag **Purity: >95% Endotoxin Level:** <1.0EU per 1µg (determined by the LAL method). **Buffer Formulation:** PBS, pH7.4, containing 0.01% Sarcosyl, 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: 8.4 Predicted Molecular Mass: 18.7kDa Accurate Molecular Mass: 24kDa 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.

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[<u>USAGE</u>]

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.

[<u>SEQUENCE</u>]

MPEVERKPKI TASRKLLLKS LMLAKAKECW EQEHEEREAE KVRYLAERIP TLQTRGLSLS ALQDLCRELH AKVEVVDEER YDIEAKCLHN TREIKDLKLK VMDLKSVKKE DTEKERPVEV GDWRKNVEAM SGMEGRKKMF DAAKSPTSQ

[ACTIVITY]

Troponin I Type 1, Slow Skeletal (TNNI1) is a slow-twitch skeletal muscle-specific isoform of troponin I, a key regulatory protein in the troponin complex. It inhibits actin-myosin interaction in the absence of calcium by binding actin and tropomyosin. Upon calcium binding to troponin C, TNNI1 undergoes conformational changes, releasing its inhibitory effect and enabling muscle contraction. TNNI1 is essential for the regulation of slow, sustained muscle contractions in type I muscle fibers.Besides,Troponin T Type 3, Fast Skeletal (TNNT3) has been identified as an interactor of TNNI1, thus a functional binding ELISA assay was conducted to detect the interaction of recombinant human TNNI1 and recombinant human TNNT3. Briefly, TNNT3 was diluted serially in PBS with 0.01% BSA (pH 7.4). Duplicate samples of 100 μ I were then transferred to

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TNNI1-coated microtiter wells and incubated for 1h at 37 $^{\circ}$ C. Wells were washed with PBST and incubated for 1h with anti-TNNT3 pAb, then aspirated and washed 3 times. After incubation with HRP labelled secondary antibody for 1h at 37 $^{\circ}$ C, wells were aspirated and washed 5 times. With the addition of substrate solution, wells were incubated 15-25 minutes at 37 $^{\circ}$ C. Finally, add 50 µL stop solution to the wells and read at 450/630nm immediately. Measured by its binding ability in a functional ELISA. When Recombinant TNNI1 is Immobilized at 2 ug/mL(100 uLwell), the concentration of TNNT3 that produces 50% optimal bindingresponse is found to be approximately 0.107 ug/mL.

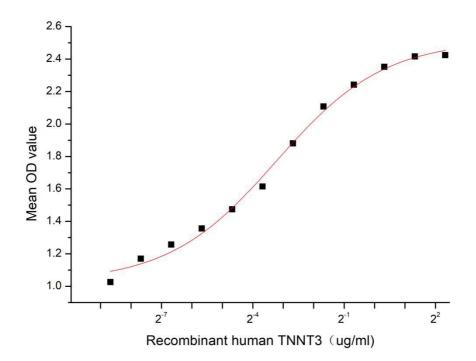


Figure 1. The binding activity of recombinant human TNNI1 and recombinant human TNNT3

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[IDENTIFICATION]

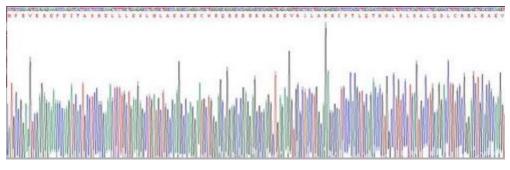


Figure 2. Gene Sequencing (extract)

	kDa 70
	44
	33
	26
-	22
	18
	14
	10

Figure 3. SDS-PAGE

Sample: Active recombinant TNNI1, 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.