

APN576Hu01 50μg

Active Fibronectin Type III Domain Containing Protein 5 (FNDC5)

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: Leu2~lle137
Tags: N-terminal His-tag

**Purity: >85%** 

**Endotoxin Level:** <1.0EU per 1μg (determined by the LAL method). **Buffer Formulation:** PBS, pH7.4, containing 0.01% SKL, 5% Trehalose.

Original Concentration: 50µg/mL

**Applications:** Cell culture; Activity Assays; In vivo assays.

(May be suitable for use in other assays to be determined by the end user.)

Predicted isoelectric point: 6.2

Predicted Molecular Mass: 19.0kDa

Accurate Molecular Mass: 44/40/23/19kDa 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 ddH<sub>2</sub>O to a concentration of 0.1-0.5 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]

LRFIQEVNT TTRSCALWDL EEDTEYIVHV QAISIQGQSP ASEPVLFKTP REAEKMASKN KDEVTMKEMG RNQQLRTGEV LIIVVVLFMW AGVIALFCRQ YDIIKDNEPN NNKEKTKSAS ETSTPEHOGG GLLRSKI

#### [ACTIVITY]

Fibronectin type III domain-containing protein 5, the precursor of irisin, is a protein that is encoded by the FNDC5 gene. FNDC5 is a 12 kDa glycosylated polypeptide hormone that regulate glycolipid metabolism, cardiovascular homeostasis metabolism, stem cell differentiation, and neuronal development. Myostatin (MSTN) has been identified as an interactor of FNDC5, thus a functional binding ELISA assay was conducted to detect the interaction of recombinant human FNDC5 and recombinant human MSTN. Briefly, FNDC5 was diluted serially in PBS with 0.01% BSA (pH 7.4). Duplicate samples of 100  $\mu l$  were then transferred to MSTN-coated microtiter wells and incubated for 1h at 37°C. Wells were washed with PBST and incubated for 1h with anti-FNDC5 pAb, then aspirated and washed 3 times. After incubation with HRP labelled secondary antibody for 1h at 37°C, wells were aspirated and washed 5 times.

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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 450/630 nm immediately. The binding activity of recombinant human FNDC5 and recombinant human MSTN was shown in Figure 1, the EC50 for this effect is 0.03 ug/mL.

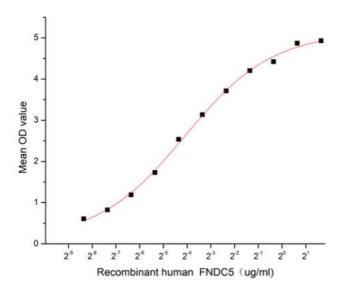


Figure 1. The binding activity of recombinant human FNDC5 and recombinant human MSTN

## [IDENTIFICATION]

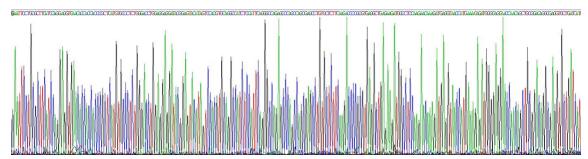


Figure 2. Gene Sequencing (extract)

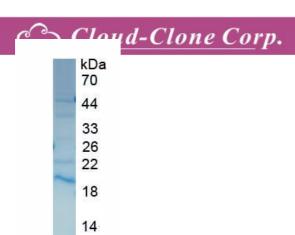


Figure 3. SDS-PAGE

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Sample: Active recombinant FNDC5, 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.