

APN576Hu01 100µg

Active Fibronectin Type III Domain Containing Protein 5 (FNDC5)

Organism Species: Homo sapiens (Human)

Instruction manual

FOR IN VITRO USE AND RESEARCH USE ONLY

NOT FOR USE IN CLINICAL DIAGNOSTIC PROCEDURES

1st Edition (Apr, 2016)

[PROPERTIES]

Source: Prokaryotic expression.

Host: *E. coli*

Residues: Leu2~Ile137

Tags: N-terminal His-tag

Purity: >92%

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

Buffer Formulation: 20mM Tris, 150mM NaCl, pH8.0, containing 1mM EDTA, 1mM DTT, 0.01% sarcosyl, 5% trehalose, and Proclin300.

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: 16.6kDa

Accurate Molecular Mass: 18kDa as determined by SDS-PAGE reducing conditions.

[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]

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LRFIQEVNT TTRSCALWDL EEDTEYIVHV QAISIQQSP ASEPVLFKTP  
REAEMASKN KDEVTMKEMG RNQQLRTGEV LIIVVVLFMW AGVIALFCRQ  
YDIIKDNEPN NNKEKTKSAS ETSTPEHQGG GLLRSKI
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[ACTIVITY]

The protein encoded by FNDC5 can be cleaved into Irisin, which is a myokine linked to exercise and lean body mass. It was reported that FNDC5 significantly decreased cell number, migration and viability through apoptosis in malignant MDA-MB-231 cells. Thus MDA-MB-231 cells were seeded overnight at a density of 5,000 cells/well, and treated with or without various concentrations of FNDC5 for 48h, then MDA-MB-231 cells were observed by inverted microscope and cell viability was measured by Cell Counting Kit-8 (CCK-8). Briefly, 10μL of CCK-8 solution was added to each well of the plate, then measure the absorbance at 450nm using a microplate reader after incubating the plate for 1-4 hours in at 37°C.

Cell apoptosis of MDA-MB-231 cells after incubation with FNDC5 for 48h observed by inverted microscope was shown in Figure 1.



A



B

Figure 1. Cell apoptosis of MDA-MB-231 cells after stimulated with FNDC5 .

(A) MDA-MB-231 cells cultured in DMEM, stimulated with 4nM FNDC5 for 48h;

(B) Unstimulated MDA-MB-231 cells cultured in DMEM for 48h.

Cell viability was assessed by CCK-8 (Cell Counting Kit-8) assay after incubation with various concentrations of FNDC5 for 48h. The dose-effect curve of FNDC5 was shown in Figure 2. It was obvious that FNDC5 significantly decreased cell viability of MDA-MB-231 cells. The ED50 for this effect is typically 1.72~2.70nM.

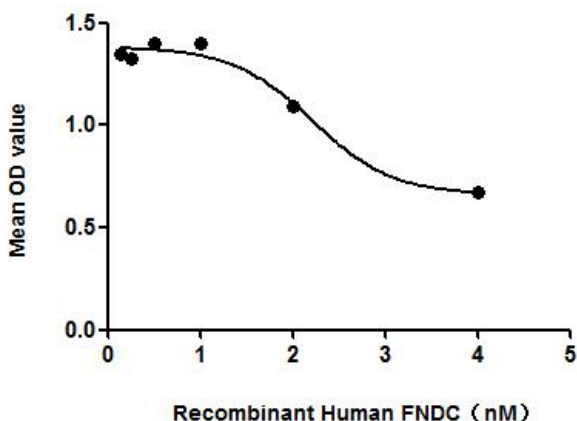


Figure 2. The dose-effect curve of FNDC5 on MDA-MB-231 cells

[IDENTIFICATION]

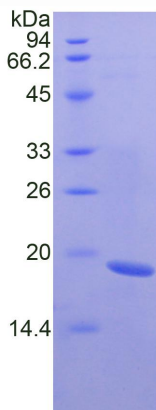


Figure 3. SDS-PAGE

Sample: Active recombinant FNDC5, Human

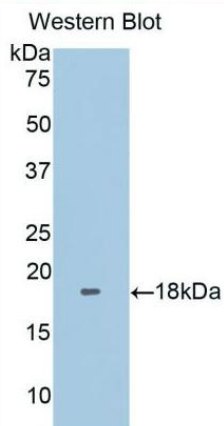


Figure 4. Western Blot

Sample: Recombinant FNDC5, Human;

Antibody: Rabbit Anti-Human FNDC5 Ab (PAN576Hu01)