APN576Hu61 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

1st Edition (Apr, 2016)

[PROPERTIES]

Source: Eukaryotic expression.

Host: 293F cell

Residues: Asp32~Glu143

Tags: N-terminal His-tag

Purity: >98%

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

Buffer Formulation: PBS, pH7.6, containing 5% trehalose.

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: 5.0

Predicted Molecular Mass: 14.2kDa

Accurate Molecular Mass: 27kDa 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 PBS (pH7.6) 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.

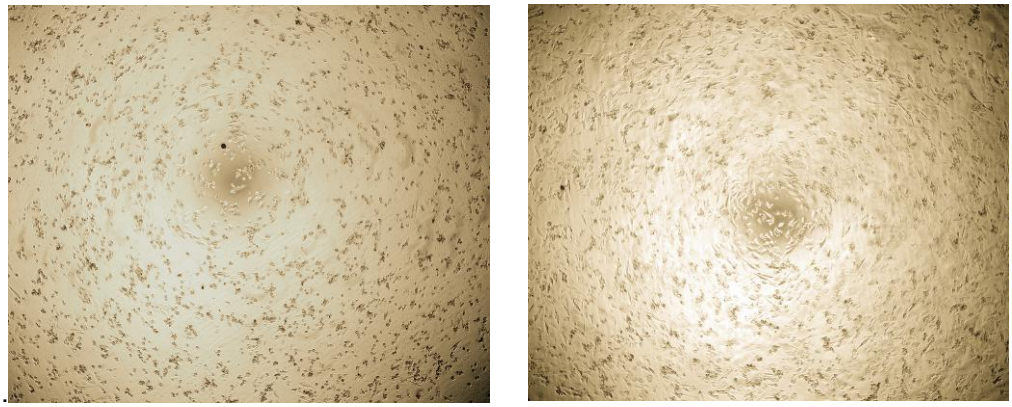
[SEQUENCE]

DSPSAPVNV TVRHLKANSA
VVSWDVLEDE VVIGFAISQQ KKDVRMLRFI QEVNTTTRSC ALWDLEEDTE
YIVHVQAISI QGQSPASEPV LFKTPREAEK MASKNKDEVT MKE

[ACTIVITY]

Fibronectin type III domain-containing protein 5, the precursor of irisin, is a protein that is encoded by the FNDC5 gene. 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 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.

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 1.35~12.52ug/mL.

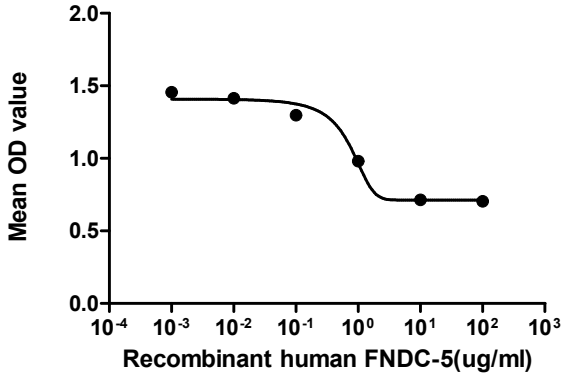


Figure 2. The dose-effect curve of FNDC5 on MDA-MB-231 cells.

[IDENTIFICATION]

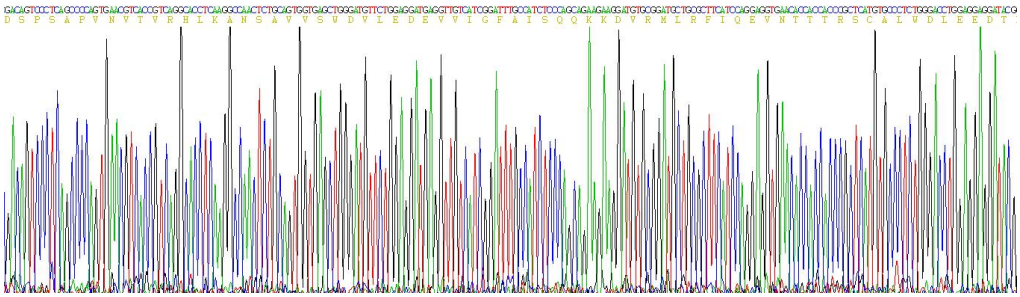


Figure 3. Gene Sequencing (extract)

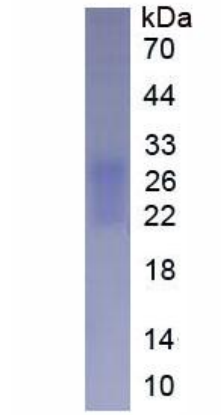


Figure 4. SDS-PAGE

Sample: Active recombinant FNDC5, Human

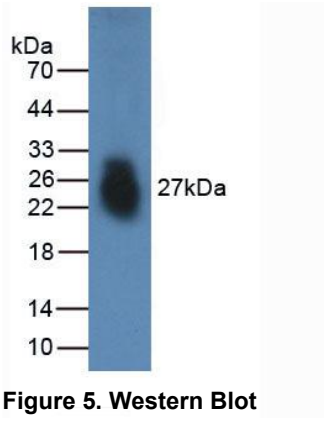


Figure 5. Western Blot

Sample: Recombinant FNDC5, Human;

Antibody: Rabbit Anti-Human FNDC5 Ab (PAN576Hu06)