#### APN576Mu61 50μg

#### Active Fibronectin Type III Domain Containing Protein 5 (FNDC5)

Organism Species: Mus musculus (Mouse)

Instruction manual

FOR RESEARCH USE ONLY
NOT FOR USE IN CLINICAL DIAGNOSTIC PROCEDURES

1th Edition (Apr, 2016)

#### [PROPERTIES]

**Source:** Eukaryotic expression.

Host: 293F cell

Residues: Asp29~Glu140 Tags: N-terminal His-tag

**Purity: >98%** 

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

**Buffer Formulation:** PBS, pH7.4, 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: 4.7

Predicted Molecular Mass: 14.2kDa

Accurate Molecular Mass: 22-30kDa 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 0.1-1.0 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]

DS PSAPVNVTVR HLKANSAVVS WDVLEDEVVI GFAISQQKKD VRMLRFIQEV NTTTRSCALW DLEEDTEYIV HVQAISIQGQ SPASEPVLFK TPREAEKMAS KNKDEVTMKE

# [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,  $10\mu L$  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  $37^{\circ}C$ 

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



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

- (A) MDA-MB-231 cells cultured in DMEM, stimulated with FNDC5(10ug/ml) 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 3.038~16.242ug/ml.

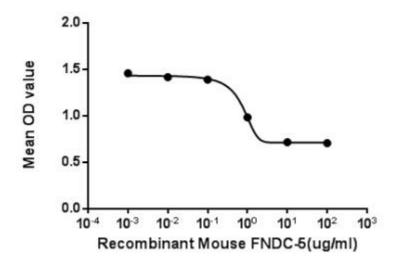


Figure 2. The dose-effect curve of FNDC5 on MDA-MB-231 cells

# [ IDENTIFICATION ]

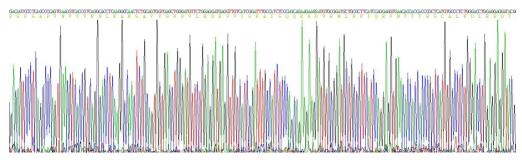


Figure 3. Gene Sequencing (extract)

kDa 70
44
33
26
22
18
14
10

Figure 4. SDS-PAGE

Sample: Active recombinant FNDC5, Mouse

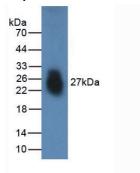


Figure 5. Western Blot

Sample: Recombinant FNDC5, Mouse;

Antibody: Rabbit Anti- Mouse FNDC5 Ab (PAN576Mu06)

# [ 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.