APN576Mu01 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

1st Edition (Apr, 2016)

[PROPERTIES]

Source: Prokaryotic expression. Host: *E. coli* Residues: Leu2~IIe137 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 0.01% skl and 5% trehalose. Applications: Cell culture; Activity Assays. (May be suitable for use in other assays to be determined by the end user.) Predicted isoelectric point: 5.8 Predicted Molecular Mass: 16.7kDa Accurate Molecular Mass: 18kDa as determined by SDS-PAGE reducing conditions. [USAGE] Percenstitute in 20mM Tris, 150mM NaCl (nH8.0) to a concentration of 0.1-10

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.

[<u>SEQUENCE</u>]

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

[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.



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.

[IDENTIFICATION]

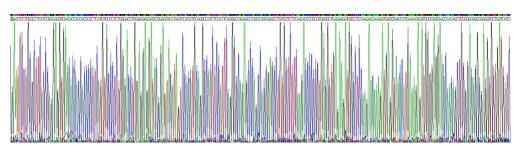


Figure2 . Gene Sequencing (extract)

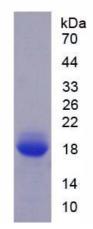


Figure3. SDS-PAGE

Sample: Active recombinant FNDC5, Mouse

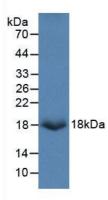


Figure4. Western Blot

Sample: Recombinant FNDC5, Mouse;

Antibody: Rabbit Anti-Mouse FNDC5 Ab (PAN576Mu01)

[IMPORTANT NOTE]

The kit is designed for in vitro and research use only, we will not be responsible for any issue if the kit was used in clinical diagnostic or any other procedures.