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APD152Ra04 100µg Active Fibulin 2 (FBLN2) Organism Species: *Rattus norvegicus (Rat) 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: Asp1014~Pro1222 Tags: N-terminal His-tag Purity: >95% Endotoxin Level: <1.0EU per 1µg (determined by the LAL method). Buffer Formulation: PBS, pH7.4, containing 0.01% SKL, 5%Trehalose . Original Concentration: 200µg/mL Applications: Cell culture; Activity Assays. (May be suitable for use in other assays to be determined by the end user.) Predicted isoelectric point: 6.0 Predicted Molecular Mass: 26.9kDa Accurate Molecular Mass: 27kDa as determined by SDS-PAGE reducing conditions.

[<u>USAGE</u>]

Reconstitute in 10mM PBS (pH7.4) 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.

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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]

DGHTCTDIDECAQGAGILCTFRCVNVPGSYQCACPEQGYTMMANGRSCKDLDECALGTHNCSEAETCHNIQGSFRCLRFDCP PNYVRVSETKCERTTCQDISECQTSPARITHYQLNFQTGLLVPAHIFRIGPAPAFAGDTISLTITKGNEEGYFVTRRLNAYT GVVSLQRSVLEPRDFALDVEMKLWRQGSVTTFLAKMYIFFTTVAP

[ACTIVITY]

Fibulin 2 (FBLN2) is the second largest member of the seven-member fibulin family of extracellular membrane (ECM) glycoproteins. The protein is known to form disulfide-linked homodimers, but it can also be secreted as an oligomer. Fibulin 2 is considered a multifunctional binding protein due to its association with numerous ECM components, but its specific interactions have yet to be determined. Fibulin 2 is localized at the interface between microfibrils and the elastin core and its known interactions include nidogen-1, perlecan, laminin, aggrecan, endostatins, versican, collagen, and tropoelastin. It is down-regulated in numerous forms of cancer including breast, colorectal, lung, esophageal, and squamous cell carcinoma, but over-expression has been shown with solid tumors. To test the effect of FBLN2 on cell apoptosis, A549 cells were seeded into triplicate wells of 96-well plates at a density of 4,000 cells/well and allowed to attach overnight, then the medium was replaced with various concentrations of recombinant rat FBLN2 diluted with 5% serum standard DMEM. After incubated for 72h, cells were observed by inverted microscope and cell proliferation was measured by Cell Counting Kit-8 (CCK-8). Briefly, 10 µl of CCK-8 solution was added to each well of the plate, then the absorbance at 450 nm was measured using a microplate reader after incubating the plate for 1h at 37 °C. Proliferation of

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A549 cells after incubation with FBLN2 for 72h observed by inverted microscope was shown in Figure 1. Cell viability was assessed by CCK-8 (Cell Counting Kit-8) assay after incubation with recombinant rat FBLN2 for 72h. The result was shown in Figure 2. It was obvious that FBLN2 significantly inhibit cell viability of A549 cells. The ED50 is 0.97 μ g/mL.



Figure 1. Inhibition of A549 cells proliferation after stimulated with FBLN2

(A) A549 cells cultured in DMEM, stimulated with 1.25 μ g/ml FBLN2 for 72h;

(B) Unstimulated A549 cells cultured in DMEM for 72h.



Figure 2. Inhibition of A549 cells proliferation after stimulated with FBLN2.

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[IDENTIFICATION]

Figure 3. SDS-PAGE

Sample: Active recombinant FBLN2, Rat

[<u>IMPORTANT NOTE</u>]

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.