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APC128Hu01 100µg Active Gremlin 1 (GREM1) Organism Species: *Homo sapiens* (Human) *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: Lys26~Asp184 Tags: N-terminal His-tag Purity: >98% Endotoxin Level: <1.0EU per 1µg (determined by the LAL method). Buffer Formulation: 20mM Tris, 150mM NaCl, pH8.0, containing 0.05% sarcosyl 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: 9.0 Predicted Molecular Mass: 24.9kDa Accurate Molecular Mass: 23kDa as determined by SDS-PAGE reducing conditions.

[<u>USAGE</u>]

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.

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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]

KKGSQ GAIPPPDKAQ HNDSEQTQSP QQPGSRNRGR GQGRGTAMPG EEVLESSQEA LHVTERKYLK RDWCKTQPLK QTIHEEGCNS RTIINRFCYG QCNSFYIPRH IRKEEGSFQS CSFCKPKKFT TMMVTLNCPE LQPPTKKKRV TRVKQCRCIS IDLD

[ACTIVITY]

Gremlin (GREM1) is an inhibitor in the TGF beta signaling pathway. GREM1 and other BMP antagonists are important in the survival of cancer stroma survival and proliferation in some cancers. The protein expression is found in many cancers and is thought to play important roles in uterine cervix, lung, ovary, kidney, breast, colon, pancreas, and sarcoma carcinomas. To test the effect of GREM1 on inhibit cell proliferation, 3T3 cells were seeded into triplicate wells of 96-well plates at a density of 5,000 cells/well and allowed to attach, replaced with serum-free overnight, then the medium was replaced with 2% serum standard DMEM including various concentrations of recombinant human GREM1. After incubated for 96h, cells were observed by inverted microscope and cell proliferation was measured by Cell Counting Kit-8 (CCK-8). Briefly, 10µL of CCK-8 solution was added to each well of the plate, then the absorbance at 450nm was measured using a microplate reader after incubating the plate for 1-4 hours at 37°C. Proliferation of 3T3 cells after incubation with GREM1 for 96h 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 GREM1 for 96h. The result was shown in Figure 2. It was obvious that GREM1 significantly increased cell viability of 3T3 cells.

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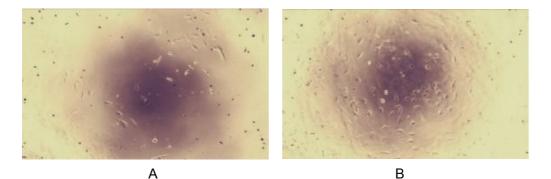
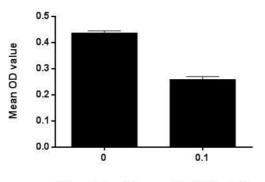


Figure 1. Inhibition of 3T3 cell proliferation after stimulated with GREM1.

(A) 3T3 cells cultured in DMEM, stimulated with 0.1ng/mL GREM1 for 96h;

(B) Unstimulated 3T3 cells cultured in DMEM for 96h.



Recombinant Human GREM1 (ng/ml)

Figure 2. Inhibition of 3T3 cell proliferation after stimulated with GREM1.

[IDENTIFICATION]

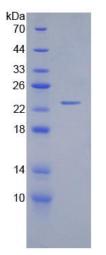


Figure 3. SDS-PAGE

Sample: Active recombinant GREM1, Human

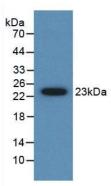


Figure 4. Western Blot

Sample: Recombinant GREM1, Human;

Antibody: Rabbit Anti-Human GREM1 Ab (PAC128Hu01)

[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.