

APC128Hu02 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: 9.6

Predicted Molecular Mass: 21.9kDa

Accurate Molecular Mass: 25kDa 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 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.

[SEQUENCE]

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KKGSQ GAIPPPDKAQ HNDSEQTQSP  
QQPGSRNRGR GQGRGTAMPG EEVLESSQEA LHVTERKYLK RDWCKTQPLK  
QTIHEEGCNS RTIINRFCYG QCNSFYIPRH IRKEEGSFQS CSFCKPKKFT  
TMMVTLNCP E LQPPTKKKRV TRVKQCRCIS IDLD
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[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. A proliferation assay was conducted to detect the bioactivity of recombinant human GREM1 using A549 cells. Briefly, A549 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 prior to the addition of various concentrations of GREM1. After incubated for 96h, cells were observed by

inverted microscope and cell proliferation was measured by Cell Counting Kit-8 (CCK-8). Briefly, 10 μ L 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 A549 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 A549 cells.

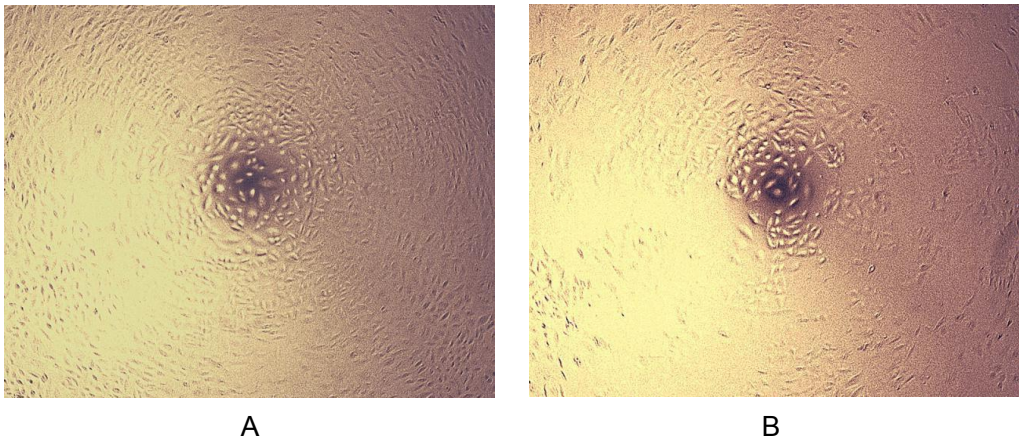


Figure 1. Cell proliferation of A549 cells after stimulated with GREM1.

(A) A549 cells cultured in DMEM, stimulated with 1ng/mL GREM1 for 96h;

(B) Unstimulated A549 cells cultured in DMEM for 96h.

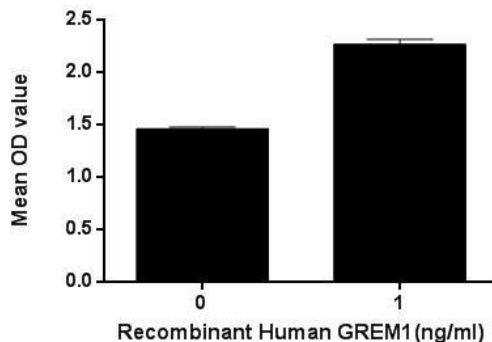


Figure 2. Cell proliferation of A549 cells after stimulated with GREM1.

[IDENTIFICATION]

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TMGAMGGSTTCMGGTGCCTCCCGGCGAGGGCCAGCCATGCTCTGGGAGCTCAGTCCGCGCGCCCTCCAGGACCCGGGAGGGGGGCGGCGGGGGCTCCCAATCCCGGGGAGGAGGCTCTGGGCTCAGCGCGAGCGGGCCCGGATGTCGAGACCGCAATACCTGGAGGGGCTGTGCAG  
K K G S Q G A I P P F D K A Q H N D S E Q T Q S P Q Q P G S R N R G R G Q G R G T A N P G E E V L E S S Q E A L H V T E R K V L K R D V C K
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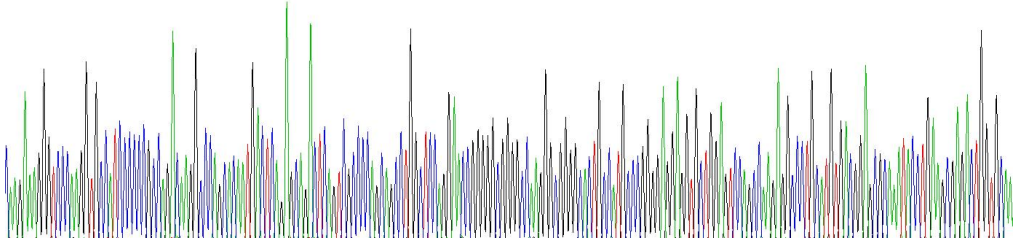


Figure 1. Gene Sequencing (extract)

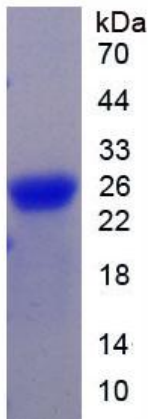


Figure 2. SDS-PAGE

Sample: Active recombinant GREM1, Human

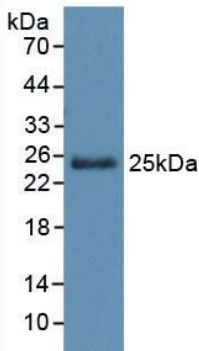


Figure 3. Western Blot

Sample: Recombinant GREM1, Human;

Antibody: Rabbit Anti-Human GREM1 Ab (PAC128Hu02)



[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.