Active Gremlin 1 (GREM1) Instruction Manual

SBPC080Ra01

Rattus norvegicus (Rat)

Buffer Formulation	100mMNaHCO3, 500mMNaCl, pH8.3, containing 0.01% SKL, 5% Trehalose.
Traits	Freeze-dried powder
Purity	> 95%
Isoelectric Point	9.6
Applications	Cell culture; Activity Assays.

ACTIVITY TEST

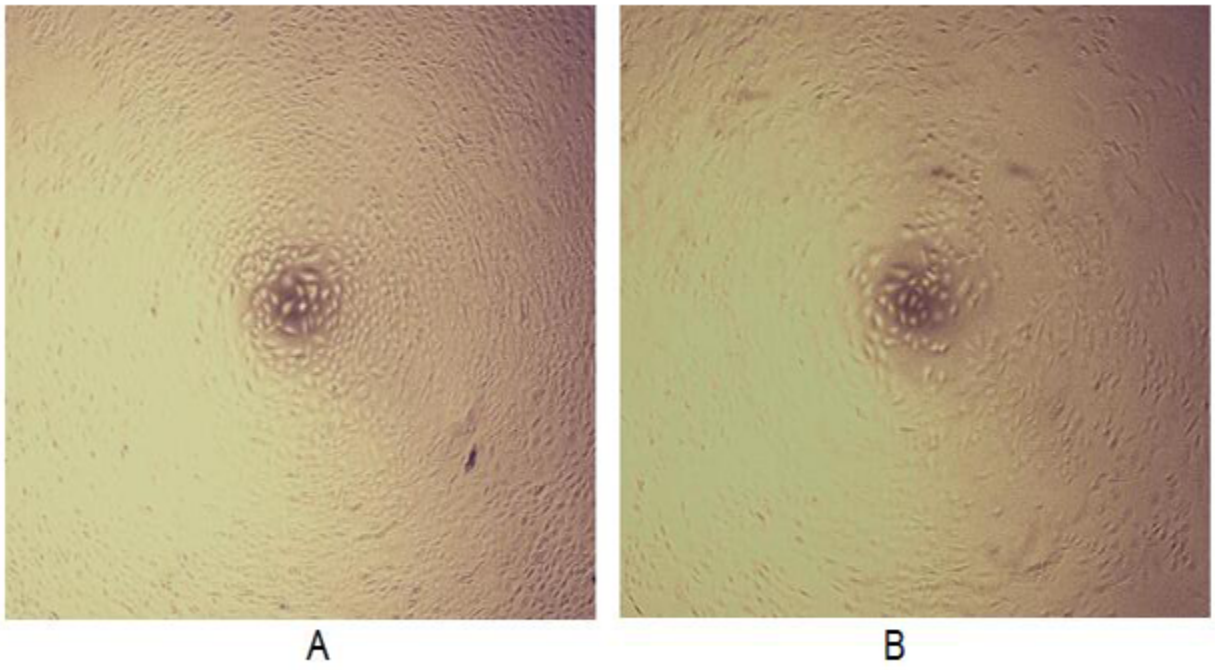

Figure 1. Cell proliferation of A549 cells after stimulated with GREM1.

Figure 1. Cell proliferation of MCF-7 cells after stimulated with GREM1.
Gremlin(GREM1) is an inhibitor in the TGF beta signaling pathway. GREM1and 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 rat 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 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 1-4 hours at 37 °C. Proliferation of A549 cells after incubation with GREM1 for 96h observed by inverted microscope was shown in Figure1. Cell viability was assessed by CCK-8(Cell Counting Kit-8) assay after incubation with recombinant GREM1 for 96h. The result was shown in Figure2. It was obvious that GREM1 significantly increased cell viability of A549 cells.(A)MCF-7 cells cultured in DMEM, stimulated with 0.001ng/ml GREM1 for 96h; (B)Unstimulated MCF7 cells cultured in DMEM for 96h.

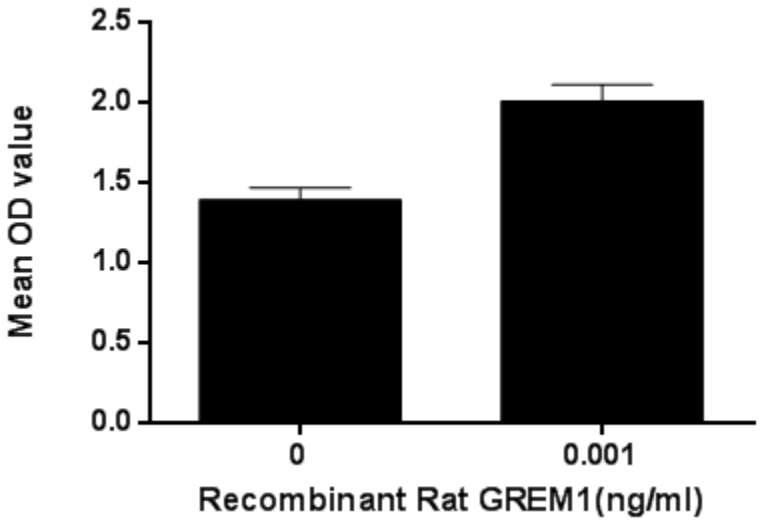

Figure. Cell proliferation of MCF-7 cells after stimulated with GREM1.

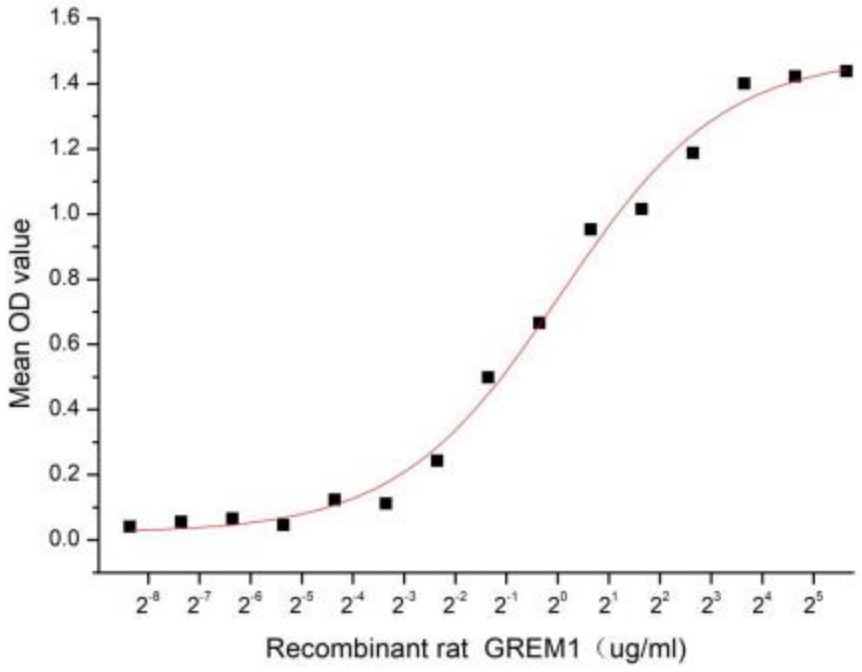

Figure 3. The binding activity of recombinant rat GREM1 and recombinant human BMP2

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. It is reported that some BMP target genes is antagonised by GREM1 and GREM2, thus a binding ELISA assay was conducted to detect the interaction of recombinant rat GREM1 and recombinant human BMP2. Briefly, GREM1 was diluted serially in PBS, with 0.01% BSA (pH 7.4). Duplicate samples of 100 µl were then transferred to BMP2-coated microtiter wells and incubated for 2h at 37℃. Wells were washed with PBST and incubated for 1h with anti-GREM1 pAb, then aspirated and washed 3 times. After incubation with HRP labelled secondary antibody, wells were aspirated and washed 5 times. With the addition of substrate solution, wells were incubated 15-25 minutes at 37℃. Finally, add 50 µL stop solution to the wells and read at 450 nm immediately. The binding activity of GREM1 and BMP2 was shown in Figure 3, the EC50 for this effect is 1.042 ug/mL.

USAGE

Reconstitute in ddH_2O to a concentration of 0.1-1.0 mg/mL. Do not vortex.

STORAGE

Avoid repeated freeze/thaw cycles. Store at 2-8°C for one month. Aliquot and store at -80°C for 12 months.

STABILITY

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.

Image

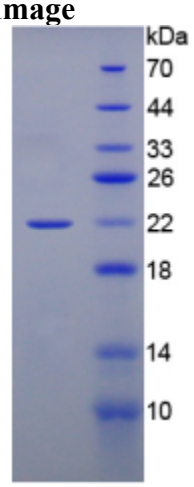

Figure. SDS-PAGE

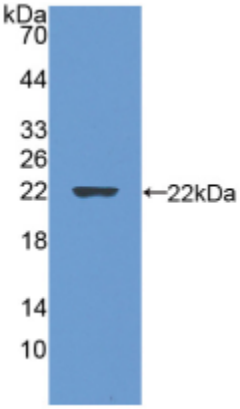

Figure. Western Blot

[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.