Active Connective Tissue Growth Factor (CTGF) Instruction Manual

SBPA006Ra01

Rattus norvegicus (Rat)

Buffer Formulation	PBS, pH7.4, containing 0.01% SKL, 5% Trehalose.
Traits	Freeze-dried powder
Purity	> 90%
Isoelectric Point	8.2
Applications	Cell culture; Activity Assays.

ACTIVITY TEST

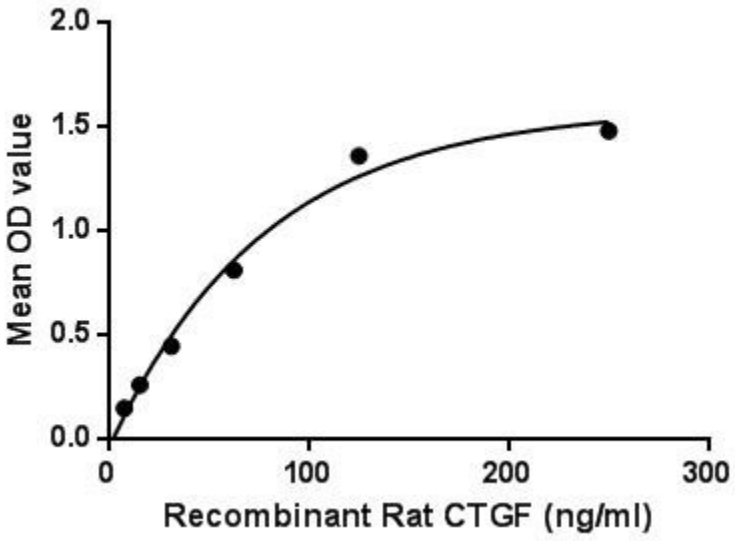

Figure. The binding activity of CTGF with ACTb.

Connective Tissue Growth Factor (CTGF), also known as CCN2 is a matricellular protein of the CCN family of extracellular matrix-associated heparin-binding proteins. CTGF has important roles in many biological processes, including cell adhesion, migration, proliferation, angiogenesis, skeletal development, and tissue wound repair, and is critically involved in fibrotic disease and several forms of cancers. Besides, Actin Beta (ACTb) has been identified as an interactor of CTGF, thus a binding ELISA assay was conducted to detect the interaction of recombinant rat CTGF and recombinant rat ACTb. Briefly, CTGF were diluted serially in PBS, with 0.01% BSA (pH 7.4). Duplicate samples of 100uL were then transferred to ACTb-coated microtiter wells and incubated for 2h at 37℃. Wells were washed with PBST and incubated for 1h with anti-CTGF pAb,

then aspirated and washed 3 times. After incubation with HRP labelled secondary antibody, wells were aspirated and washed 3 times. With the addition of substrate solution, wells were incubated 15-25 minutes at 37℃. Finally, add 50μL stop solution to the wells and read at 450nm immediately. The binding activity of of CTGF and ACTb was shown in Figure 1, and this effect was in a dose dependent manner.

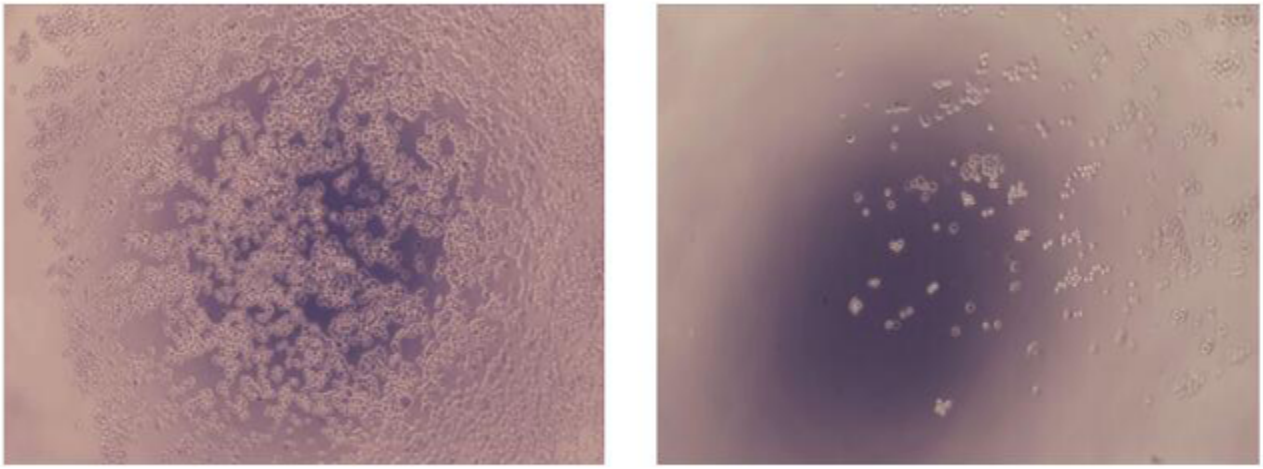

Figure.The adhere effect of CTGF on 3T3 cells.
(A) 3T3 cells were seeded into the well containting CTGF 1μg/mL and incubated for 1h at
37 ℃;
(B) 3T3 cells were seeded into the well without CTGF and incubated for 1h at 37℃;
To measure the effect of CTGF on cell adhesion, a general procedure performance as follows: 100μL PBS containing recombinant CTGF were incubated overnight at 4℃ in 96-well ELISA plates. Wells were blocked with 200μL PBS containing 3% BSA and then incubated for 1h at 37 ℃with 100μL PBS containing approximately 5x104 3T3 cells. Adherent cells were then fixed for 15 min with 5% formaldehyde and non-adherent cells were removed by washing each well three times with PBS. The remaining cells were incubated with 0.5% cristal violet for 10mins then counted at high magnification (×400) randomly (five fields for each well).

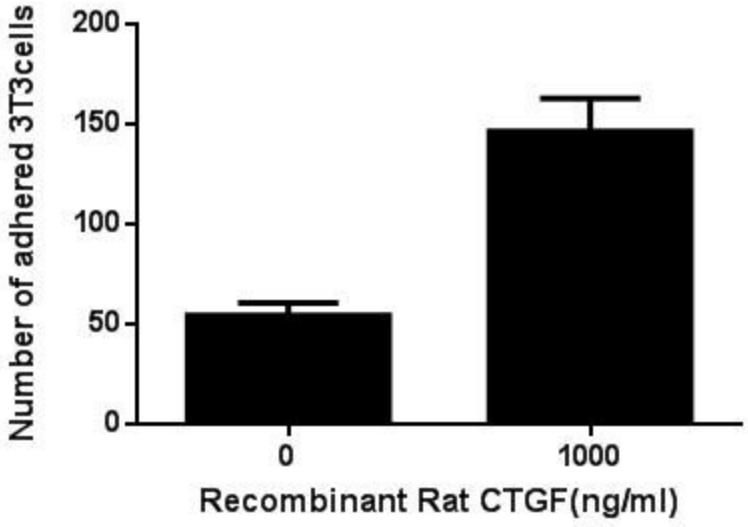

Figure.The adhere effect of CTGF on 3T3 cells.

USAGE

Reconstitute in 10mM PBS (pH7.4) 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

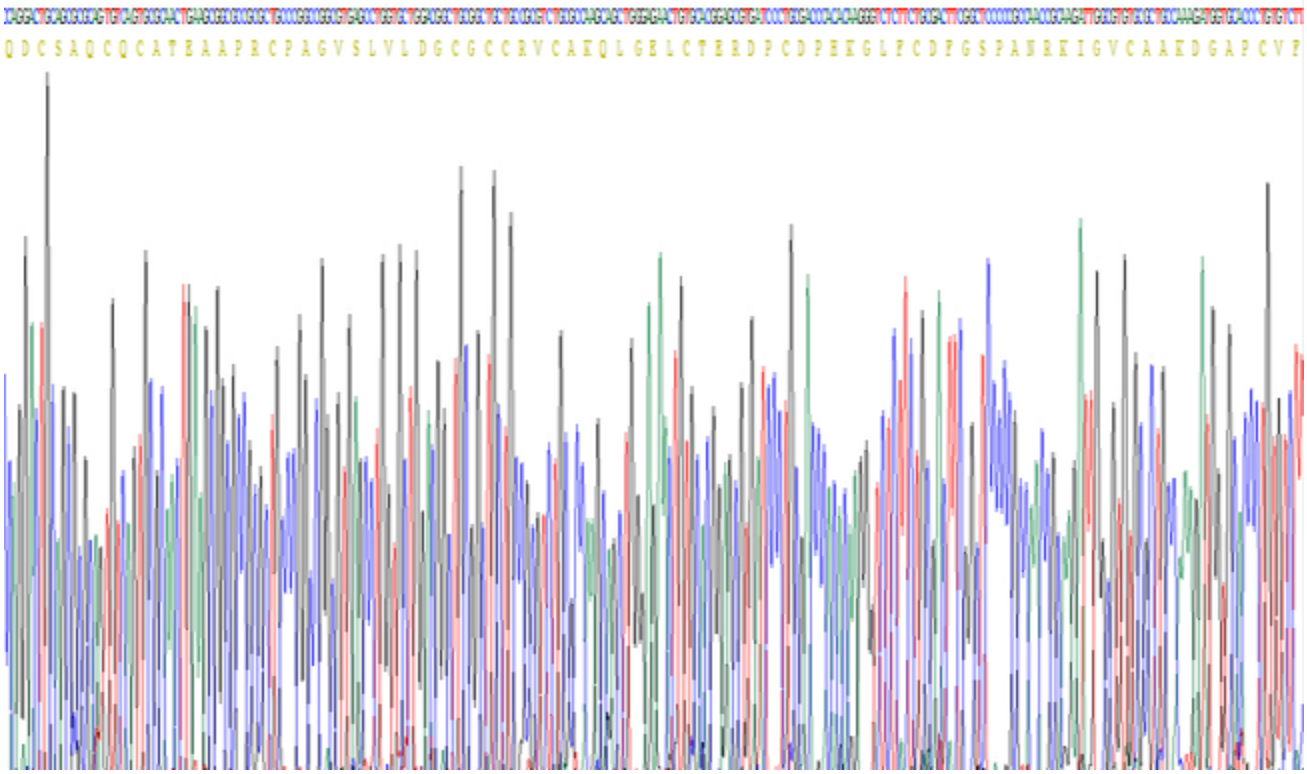

Figure. SDS-PAGE

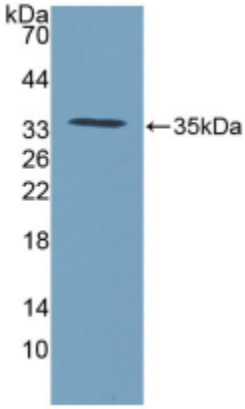

Figure. Western Blot

[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.