

APA010Ra01 50µg
Active Connective Tissue Growth Factor (CTGF)
Organism Species: *Rattus norvegicus* (Rat)
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: Gln25~Ala347

Tags: N-terminal His-tag

Purity: >92%

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: 8.2

Predicted Molecular Mass: 36.7kDa

Accurate Molecular Mass: 35kDa as determined by SDS-PAGE reducing conditions.

[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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QDCSAQ CQCAAEAAPR CPAGVSLVLD
GCGCCRVCAK QLGELCTERD PCDPHKGLFC DFGSPANRKI GVCTAKDGAP
CVFGGSVYRS GESFQSSCKY QCTCLDGAVG CVPLCSMDVR LPSPDCPFPR
RVKLP GKCE EWVCDEPKDR TVVGPALAA Y RLEDTFGPDP TMMRANCLVQ
TTEWSACSKT CGMGISTRVT NDNTFCRLEK QSRLCMVRPC EADLEENIKK
GKKCIRTPKI AKPVKFELSG CTSVKTYRAK FCGVCTDGRC CTPHRTTTL P
VEFKCPDGEI MKKNMMFIKT CACHYNCPGD NDIFESLYYR KMYGDMA
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[ACTIVITY]

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 °C . 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°C . 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.

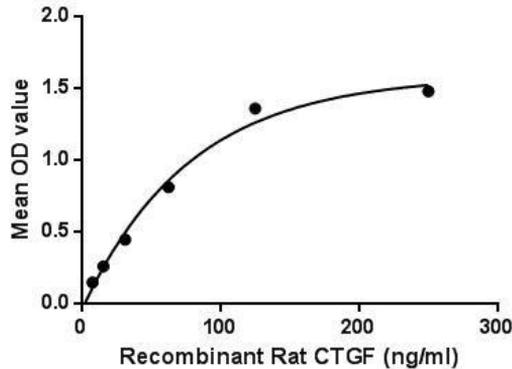
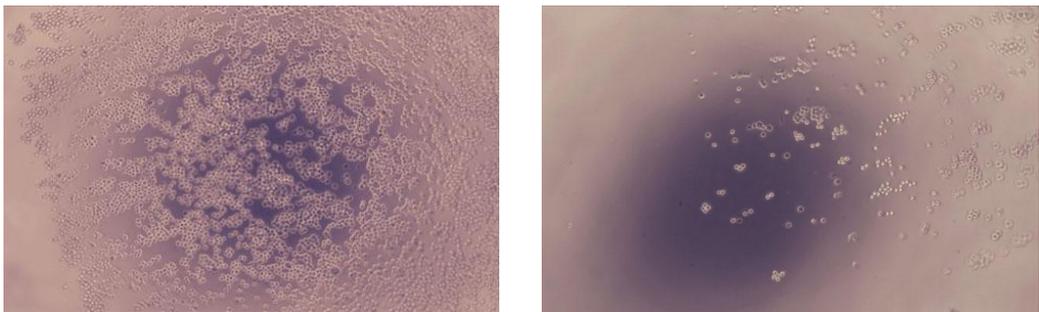


Figure 1. The binding activity of CTGF with ACTb.

To measure the effect of CTGF on cell adhesion, a general procedure performance as follows: 100 μ L PBS containing recombinant CTGF were incubated overnight at 4 $^{\circ}$ C in 96-well ELISA plates. Wells were blocked with 200 μ L PBS containing 3% BSA and then incubated for 1h at 37 $^{\circ}$ C with 100 μ L PBS containing approximately 5×10^4 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 ($\times 400$) randomly (five fields for each well).



A

B

Figure 2. The adhere effect of CTGF on 3T3 cells.

(A) 3T3 cells were seeded into the well containing CTGF 1 μ g/mL and incubated for 1h at 37 $^{\circ}$ C;

(B) 3T3 cells were seeded into the well without CTGF and incubated for 1h at 37 $^{\circ}$ C;

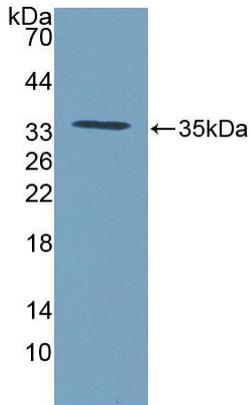


Figure 6. Western Blot

Sample: Recombinant CTGF, Rat;

Antibody: Rabbit Anti-Rat CTGF Ab (PAA010Ra01)

[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.