APA123Hu01 100μg
Active Transforming Growth Factor Alpha (TGFa)
Organism Species: Homo sapiens (Human)
Instruction manual

FOR IN VITRO USE AND RESEARCH USE ONLY
NOT FOR USE IN CLINICAL DIAGNOSTIC PROCEDURES

1th Edition (Apr, 2016)

[PROPERTIES]

Source: Prokaryotic expression.
Host: *E. coli*
Residues: Glu24~Ala98
Tags: N-terminal His-tag
Purity: >98%
Buffer Formulation: 20mM Tris, 150mM NaCl, pH8.0, containing 0.05% sarcosyl and 5% trehalose.
Applications: Cell culture; Activity Assays; In vivo assays.
(May be suitable for use in other assays to be determined by the end user.)
Predicted isoelectric point: 6.6
Predicted Molecular Mass: 11.7kDa
Accurate Molecular Mass: 15kDa 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 2-8ºC for one month.

Aliquot and store at -80ºC 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 37ºC 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.

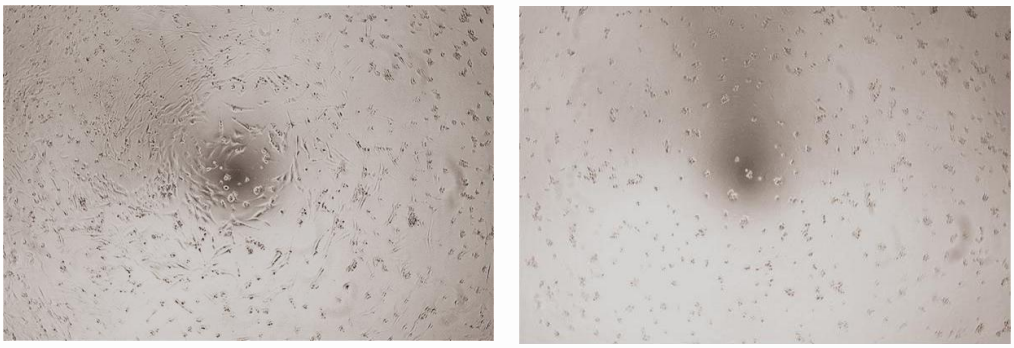
[SEQUENCE]

ENSTSPL SDPPVAAAVV SHFNDCPDSH
TQFCFHGTCR FLVQEDKPAC VCHSGYVGAR CEHADLLAVV AASQKKQA

[ACTIVITY]

Transforming growth factor alpha (TGF-α), a ligand for the epidermal growth factor receptor, which activates a signaling pathway for cell proliferation, differentiation and development. To test the effect of TGF-α on cell proliferation of 3T3 fibroblasts, 3T3 cells were seeded into triplicate wells of 96-well plates at a density of 2, 000 cells/well and allowed to attach overnight, then the medium was replaced with serum-free standard DMEM prior to the addition of various concentrations of TGF-α. After incubated for 72h, 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 measure the absorbance at 450nm using a microplate reader after incubating the plate for 1-4 hours at 37°C.

Cell proliferation of 3T3 cells after incubation with TGF-α for 72h observed by inverted microscope was shown in Figure 1.



A B

Figure 1. Cell proliferation of 3T3 cells after stimulated with TGF-α.

(A) 3T3 cells cultured in DMEM, stimulated with 1ng/mL TGF-α 72h;

(B) Unstimulated 3T3 cells cultured in serum-free DMEM for 72h.

The dose-effect curve of TGF-α was shown in Figure 2. It was obvious that TGF-α significantly promoted cell proliferation of 3T3 cells. The ED50 for this effect is typically 0.6198 to 8.210ng/mL.

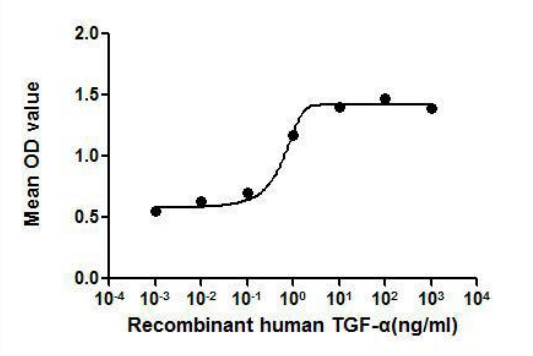


Figure 2. The dose-effect curve of TGF-α on 3T3 cells.

[IDENTIFICATION]

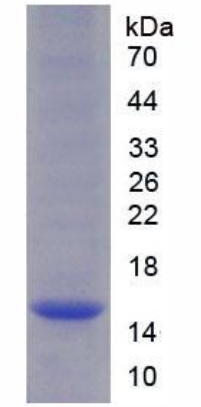


Figure 3. SDS-PAGE

Sample: Active recombinant TGFa, Human

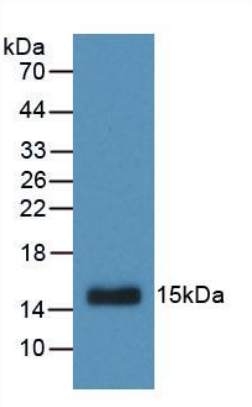


Figure 4. Western Blot

Sample: Recombinant TGFa, Human;

Antibody: Rabbit Anti-Human TGFa Ab (PAA123Hu01)