

**APA218Hu01 100µg**  
**Active Transforming Growth Factor Beta 2 (TGFβ2)**  
**Organism Species: Homo sapiens (Human)**  
***Instruction manual***

FOR RESEARCH USE ONLY  
NOT FOR USE IN CLINICAL DIAGNOSTIC PROCEDURES

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1st Edition (Apr, 2016)

## **[ PROPERTIES ]**

**Source:** Prokaryotic expression.

**Host:** *E. coli*

**Residues:** Ser303~Glu414

**Tags:** N-terminal His-tag

**Purity:** >92%

**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:** 8.9

**Predicted Molecular Mass:** 16.6kDa

**Accurate Molecular Mass:** 17kDa 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.

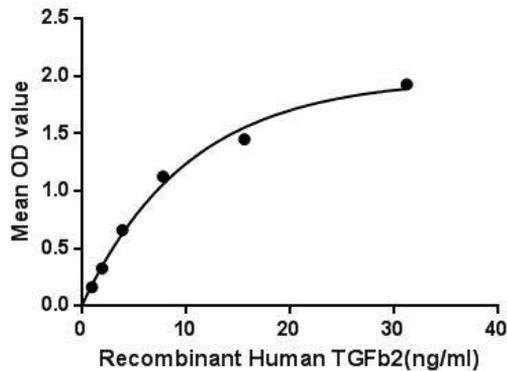
**Stability Test:** The thermal stability is described by the loss rate. The loss rate was determined by accelerated thermal degradation test, that is, incubate the protein at 37°C for 48h, and no obvious degradation and precipitation were observed. The loss rate is less than 5% within the expiration date under appropriate storage condition.

## **[ SEQUENCE ]**

SGKTPHLL LMLLPSYRLE SQQTNRKRR ALDAAYCFRN VQDNCCLRPL  
YIDFKRDLGW KWIHEPKGYN ANFCAGACPY LWSSDTQHSR VLSLYNTINP  
EASASPCCVS QDLE

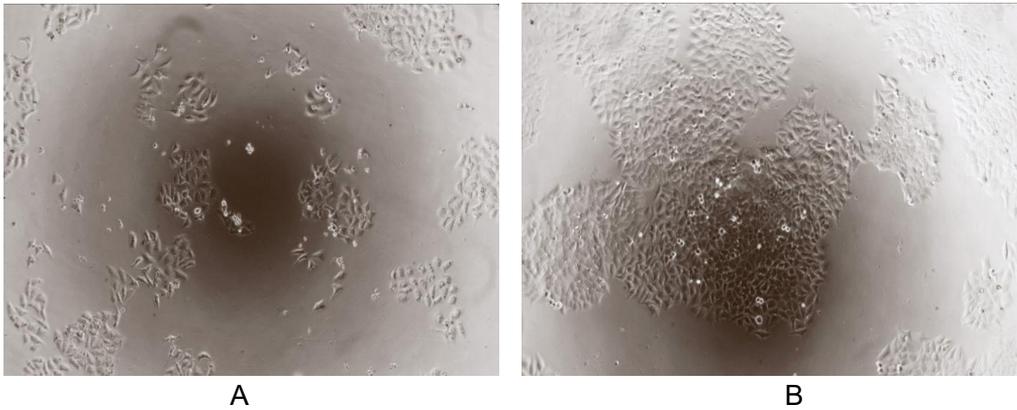
## **[ ACTIVITY ]**

Transforming growth factor-beta 2 (TGFb2) is a secreted protein known as a cytokine that performs many cellular functions and has a vital role during embryonic development. It is an extracellular glycosylated protein. It is known to suppress the effects of interleukin dependent T-cell tumors. There are two named isoforms of this protein, created by alternative splicing of the same gene. Besides, Amyloid Precursor Protein (APP) has been identified as an interactor of TGFb2, thus a binding ELISA assay was conducted to detect the interaction of recombinant human TGFb2 and recombinant human APP. Briefly, TGFb2 were diluted serially in PBS, with 0.01% BSA (pH 7.4). Duplicate samples of 100uL were then transferred to APP-coated microtiter wells and incubated for 2h at 37°C. Wells were washed with PBST and incubated for 1h with anti-TGFb2 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 TGFb2 and APP was shown in Figure 1, and this effect was in a dose dependent manner.



**Figure 1. The binding activity of TGFb2 with APP.**

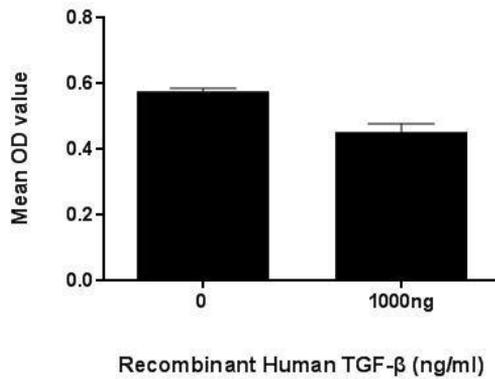
Transforming growth factor beta (TGF- $\beta$ ) is a multifunctional cytokine belonging to the transforming growth factor superfamily. The TGF- $\beta$  superfamily includes endogenous growth inhibiting proteins; an increase in expression of TGF- $\beta$  often correlates with the malignancy of many cancers and a defect in the cellular growth inhibition response to TGF- $\beta$ . Its immunosuppressive functions then come to dominate, contributing to oncogenesis. To test the effect of TGF- $\beta$  on inhibit HGF-dependent proliferation, HepG2 cells were seeded into triplicate wells of 96-well plates at a density of 2,000 cells/well and allowed to attach, replaced with serum-free overnight, then the medium was replaced with 2% serum standard DMEM including 1ng/mL HGF prior to the addition of various concentrations of recombinant human TGF- $\beta$ . After incubated for 96h, cells were observed by inverted microscope and cell proliferation was measured by Cell Counting Kit-8 (CCK-8). Briefly, 10 $\mu$ 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 $^{\circ}$ C. The inhibitory effect of TGF- $\beta$  on HGF-dependent proliferation of HepG2 cells observed by inverted microscope was shown in Figure 2. Cell viability was assessed by CCK-8 assay after incubation with recombinant TGF- $\beta$  for 96h. The result was shown in Figure 3. It was obvious that TGF- $\beta$  significantly decreased cell viability of HepG2 cells.



**Figure 2.**The inhibitory effect of TGF-β on cell proliferation of HepG2 cells.

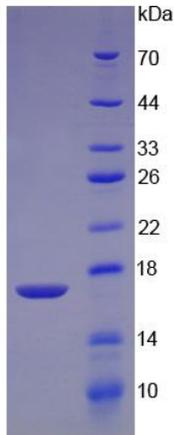
**(A)** HepG2 cells cultured in DMEM, stimulated with 1µg/mL TGF-β for 96h;

**(B)** Unstimulated HepG2 cells cultured in DMEM for 96h.



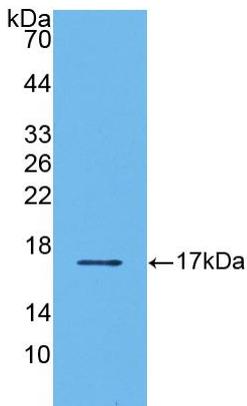
**Figure 3.** TGF-β inhibit cell proliferation of HepG2 cells.

**[ IDENTIFICATION ]**



**Figure 4. SDS-PAGE**

**Sample: Active recombinant TGFb2, Human**



**Figure 5. Western Blot**

**Sample: Recombinant TGFb2, Human;**

**Antibody: Rabbit Anti-Human TGFb2 Ab (PAA218Hu01)**

**[ 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.