**APA134Hu01 100μg**
**Active Tumor Necrosis Factor Beta (TNFb)**
**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:** Pro35~Leu205
**Tags:** N-terminal His-tag
**Purity:** >98%
**Endotoxin Level:** <1.0EU per 1μg (determined by the LAL method).
**Buffer Formulation:** 20mM Tris, 150mM NaCl, pH8.0, containing 1mM EDTA, 1mM DTT, 0.01% sarcosyl, 5% trehalose, and Proclin300.
**Predicted isoelectric point:** 9.3
**Predicted Molecular Mass:** 22.2kDa
**Accurate Molecular Mass:** 23kDa as determined by SDS-PAGE reducing conditions.
**Applications:** Cell culture; Activity Assays; In vivo assays.
(May be suitable for use in other assays to be determined by the end user.)

## [ 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.

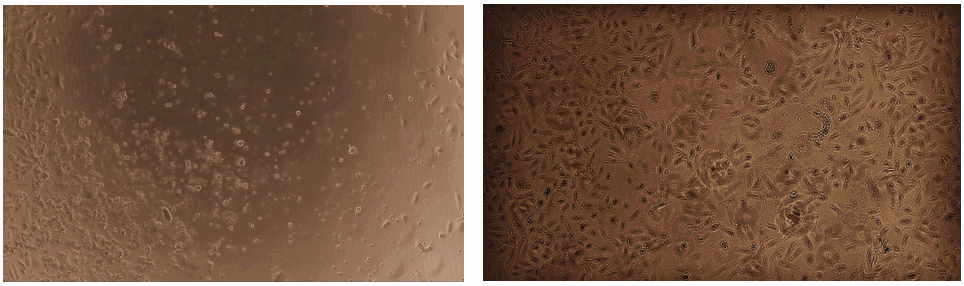
## [ SEQUENCE ]

PGVGL TPSAAQTARQ
HPKMHLAHST LKPAAHLIGD PSKQNSLLWR ANTDRAFLQD GFSLSNNSLL
VPTSGIYFVY SQVVFSGKAY SPKATSSPLY LAHEVQLFSS QYPFHVPLLS
SQKMVYPGLQ EPWLHSMYHG AAFQLTQGDQ LSTHTDGIPH LVLSPSTVFF
GAFAL

## [ ACTIVITY ]

TNF-β, a member of the tumor necrosis factor family, is a potent lymphoid factor that exerts cytotoxic effects on a wide range of tumor cells. The biological effects of TNF-β are very similar to TNF-α, due to the similarity of molecular structure and the receptors. As reported, TNF-β could inhibit the proliferation and induce necrosis of A549 cells, and the concentration of IL-1β in cell supernatant will increase after stimulation. Therefore, A549 cells were incubated in DMEM with TNF-β (10ng/mL) for 8h, 24h, 48h, 72h, then cells were observed by inverted microscope and IL-1β was detected in the cell supernatant by ELISA .

Cell necrosis after incubation with TNF-β(10ng/mL) for 72h was shown in Figure 1.



A B

**Figure 1. Effect of TNF-β on A549 cells.**

**(A) A549 cells cultured in DMEM, stimulated with 10ng/mL TNF-β for 72h;**

**(B) A549 cells cultured in DMEM for 72h.**

The production of IL-1β after incubation with TNF-β (10ng/mL) for 8h is shown in Table 1.

**Table 1. Effect of TNF-β on A549 cells by ELISA.**

| Sample (cell supernatant of A549 cells) | Concentration of IL-1β (ng/mL) |
|---|---|
| Stimulated with TNF-β (10ng/mL) | 95.9 |
| Unstimulated | 4.9 |

## [ IDENTIFICATION ]

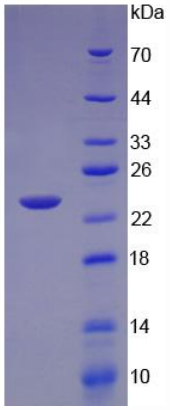



**Figure 2. SDS-PAGE**

**Sample: Active recombinant TNFb, Human**

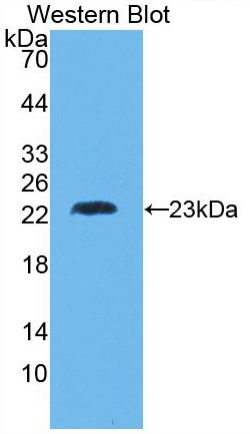



**Figure 3. Western Blot**

**Sample: Recombinant TNFb, Human;**

**Antibody: Rabbit Anti-Human TNFb Ab (PAA134Hu01)**