APB499Hu01 100µg Active Tumor Necrosis Factor Receptor Superfamily, Member 1A (TNFRSF1A) Organism Species: *Homo sapiens* (Human) *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: Thr60~Tyr236 Tags: Two N-terminal Tags, His-tag and GST-tag Purity: >95% 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: 6.4

Predicted Molecular Mass: 49.9kDa

Accurate Molecular Mass: 50kDa as determined by SDS-PAGE reducing conditions.

[<u>USAGE</u>]

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.

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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]

T KCHKGTYLYN DCPGPGQDTD CRECESGSFT ASENHLRHCL SCSKCRKEMG QVEISSCTVD RDTVCGCRKN QYRHYWSENL FQCFNCSLCL NGTVHLSCQE KQNTVCTCHA GFFLRENECV SCSNCKKSLE CTKLCLPQIE NVKGTEDSGT TVLLPLVIFF GLCLLSLLFI GLMYRY

[ACTIVITY]

Tumor necrosis factor receptor superfamily member 1B (TNFRSF1B), also known as tumor necrosis factor receptor 2 (TNFR2) and CD120b, is a membrane receptor that binds tumor necrosis factor-alpha (TNF α). This protein and TNF-receptor 1 form a heterocomplex that mediates the recruitment of two anti-apoptotic proteins, c-IAP1 and c-IAP2, which possess E3 ubiquitin ligase activity. TNFRSF1B can inhibit cell apoptosis which induced by TNFα. Briefly, A549 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 1µg/mL TNFa and various concentrations of recombinant human TNFRSF1B. After incubated for 96h, 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 the absorbance at 450nm was measured using a microplate reader after incubating the plate at 37°C for 1-4 hours. Apoptosis of A549 cells had been inhibit after incubation with TNFRSF1B for 96h observed by inverted microscope was shown in Figure 1. Cell viability was assessed by CCK-8 (Cell Counting Kit-8) assay after incubation with recombinant

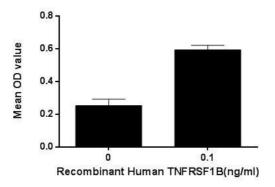
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TNFRSF1B for 96h. The result was shown in Figure 2. It was obvious that TNFRSF1B significantly suppress cell apoptosis induced by TNF α .



Figure 1. The apoptosis of A549 cells by TNF α was inhibited by TNFRSF1B.

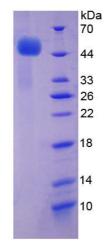
- (A) A549 cells cultured in DMEM contain 1μ g/mL TNF α and 0.1ng/mL TNFRSF1B for 96h;
- (B) A549 cells cultured in DMEM only contain 1µg/mL TNFα for 96h.

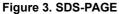






[IDENTIFICATION]





Sample: Active recombinant TNFRSF1A, Human

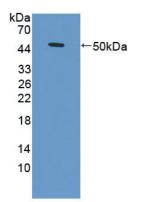


Figure 4. Western Blot Sample: Recombinant TNFRSF1A, Human; Antibody: Rabbit Anti-Human TNFRSF1A Ab (PAB499Hu01)

[<u>IMPORTANT NOTE</u>]

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.