

Human TNF Receptor II protein, His tag (active)

Cat. No. GTX00235-pro

Application	Functional Assay
Species	Human

Package
10 µg

APPLICATION

Application Note

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- α (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. Besides, Tumor Necrosis Factor Alpha (TNF α) has been identified as an interactor of TNFRSF1B, thus a binding ELISA assay was conducted to detect the interaction of recombinant human TNFRSF1B and recombinant human TNF α . Briefly, TNFRSF1B were diluted serially in PBS, with 0.01% BSA (pH 7.4). Duplicate samples of 100 µl were then transferred to TNF α -coated microtiter wells and incubated for 2h at 37°C. Wells were washed with PBST and incubated for 1h with anti-TNFRSF1B 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 50 µl stop solution to the wells and read at 450nm immediately. The binding activity of TNFRSF1B and TNF α was in a dose dependent manner.

To tested cell apoptosis, A549 cells were seeded into triplicate wells of 96-well plates at a density of 2000 cells/well. and allowed to attach, replaced with serum-free overnight, then the medium was replaced with 2% serum standard DMEM including 1µg/ml TNF α 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, 10 µl 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. Cell viability was assessed by CCK-8 (Cell Counting Kit-8) assay after incubation with recombinant TNFRSF1B for 96h. And TNFRSF1B significantly suppress cell apoptosis induced by TNF α .

Observed MW 50 kDa.

PROPERTIES

Form	Lyophilized powder
Buffer	Reconstitute with 20mM Tris and 150mM NaCl to 0.1-1.0mg/ml. Do not vortex. Lyophilized from 20mM Tris, 150mM NaCl, 1mM EDTA, 1mM DTT, 0.01% SKL, 5% Trehalose.
Preservative	ProClin 300
Storage	For short-term storage (1-2 weeks), store at 4°C. For long-term storage, store at -20°C or below. After reconstitution, keep as concentrated solution. Avoid freeze-thaw cycles.
Region/Sequence	N-terminal His-Tag; Ala298~Leu441 (NP_001057.1)
Expression System	E. coli
Purity	> 95%
Endotoxin	< 1 EU/µg
Conjugation	Unconjugated

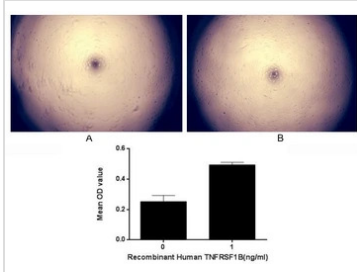


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Note

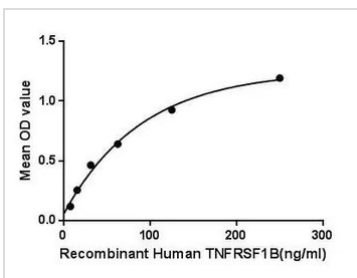
For laboratory use only. Not for any clinical, therapeutic, or diagnostic use in humans or animals. Not for animal or human consumption.

DATA IMAGES

GTX00235-pro Functional Assay Image

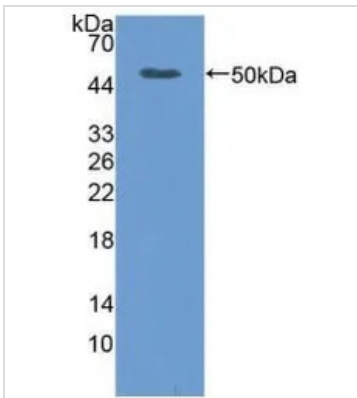
The suppression effect of GTX00235-pro Human TNF Receptor II protein (active) on TNF alpha induced apoptosis.

(A) A549 cells cultured in DMEM only contain 1 µg/ml TNF alpha for 96hrs.

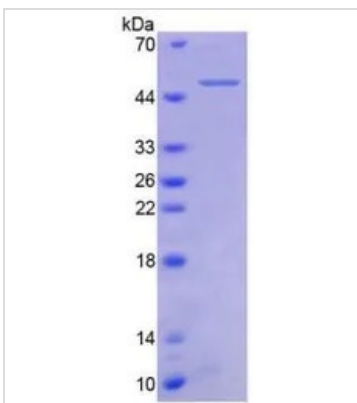
(B) A549 cells cultured in DMEM contain 1 µg/ml TNF alpha and 1 ng/ml TNFRSF1B for 96hrs.


GTX00235-pro Functional Assay Image

Functional ELISA analysis of GTX00235-pro Human TNF Receptor II protein (active) which can bind immobilized TNFa protein.


GTX00235-pro Image

WB analysis of GTX00235-pro Human TNF Receptor II protein (active).


GTX00235-pro Image

SDS-PAGE analysis of GTX00235-pro Human TNF Receptor II protein (active).



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