Anti-TNF Receptor II Antibody [JM113-01]

ET1703-63

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IF-Cell, IF-Tissue, IHC-P, FC, IP, IHC-Fr
Molecular Wt:	Predicted band size: 48 kDa
Clone number:	JM113-01

Description: Tumor necrosis factor receptor 2 (TNFR2), also known as tumor necrosis factor receptor superfamily member 1B (TNFRSF1B) and CD120b, is one of two membrane receptors that binds tumor necrosis factor-alpha (TNFα). Like its counterpart, tumor necrosis factor receptor 1 (TNFR1), the extracellular region of TNFR2 consists of four cysteine-rich domains which allow for binding to TNFα. TNFR1 and TNFR2 possess different functions when bound to TNFα due to differences in their intracellular structures, such as TNFR2 lacking a death domain (DD). The protein encoded by this gene is a member of the tumor necrosis factor receptor superfamily, which also contains TNFRSF1A. 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. The function of IAPs in TNF-receptor signalling is unknown, however, c-IAP1 is thought to potentiate TNF-induced apoptosis by the ubiquitination and degradation of TNF-receptor-associated factor 2 (TRAF2), which mediates anti-apoptotic signals. Knockout studies in mice also suggest a role of this protein in protecting neurons from apoptosis by stimulating antioxidative pathways.

Immunogen:	Synthetic peptide within Human TNF Receptor II aa 426-461 / 461.
Positive control:	THP-1 cell lysate, Jurkat cell lysate, K-562 cell lysate, RAW264.7 cell lysate, PC-12 cell lysate, Mouse spleen tissue lysate, human uterus tissue, HL-60, RAW264.7, PC-12.
Subcellular location:	Cell membrane, Membrane, Secreted.
Database links:	SwissProt: P20333 Human | P25119 Mouse | Q80WY6 Rat
Recommended Dilutions:	
WB	1:2,000
IF-Cell	1:50-1:200
IF-Tissue	1:50-1:200
IHC-P	1:50-1:200
FC	1:50-1:100
IP	Use at an assay dependent concentration.
IHC-Fr	1:200
Storage Buffer:	1*TBS (pH7.4), 0.05% BSA, 40% Glycerol. Preservative: 0.05% Sodium Azide.
Storage Instruction:	Shipped at 4℃. Store at +4℃ short term (1-2 weeks). It is recommended to aliquot into single-use upon delivery. Store at -20℃ long term.
Purity:	Protein A affinity purified.

Images

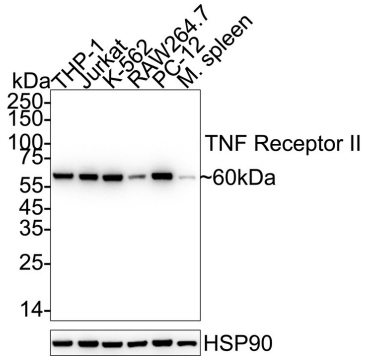

Fig1: Western blot analysis of TNF Receptor II on different lysates with Rabbit anti-TNF Receptor II antibody (ET1703-63) at 1/2,000 dilution.

Lane 1: THP-1 cell lysate (20 µg/Lane)
Lane 2: Jurkat cell lysate (20 µg/Lane)
Lane 3: K-562 cell lysate (20 µg/Lane)
Lane 4: RAW264.7 cell lysate (20 µg/Lane)
Lane 5: PC-12 cell lysate (20 µg/Lane)
Lane 6: Mouse spleen tissue lysate (40 µg/Lane)

Predicted band size: 48 kDa
Observed band size: 60 kDa

Exposure time: 10 seconds; ECL: K1801;

4-20% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (ET1703-63) at 1/2,000 dilution was used in 5% NFDM/TBST at 4℃ overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

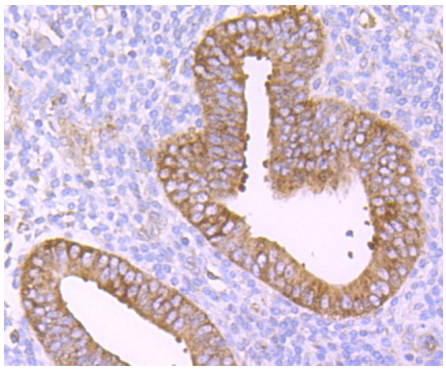

Fig2: Immunohistochemical analysis of paraffin-embedded human uterus tissue using anti-TNF Receptor II antibody. The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 8.0-8.4) for 20 minutes.The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1703-63, 1/50) for 30 minutes at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

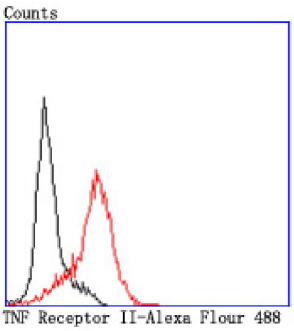

Fig3: Flow cytometric analysis of TNF Receptor II was done on HL-60 cells. The cells were fixed, permeabilized and stained with the primary antibody (ET1703-63, 1/50) (red). After incubation of the primary antibody at room temperature for an hour, the cells were stained with a Alexa Fluor 488-conjugated Goat anti-Rabbit IgG Secondary antibody at 1/1000 dilution for 30 minutes.Unlabelled sample was used as a control (cells without incubation with primary antibody; black).

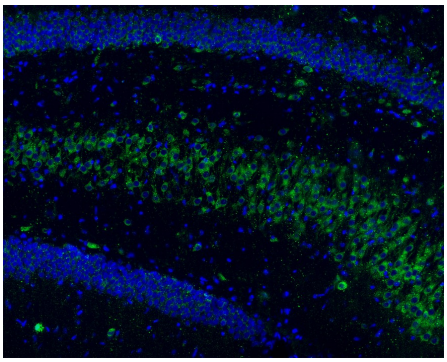

Fig4: Immunofluorescence analysis of frozen mouse hippocampus tissue labeling TNF Receptor II with Rabbit anti-TNF Receptor II antibody (ET1703-63).

The tissues were blocked in 3% BSA for 30 minutes at room temperature, washed with PBS, and then probed with the primary antibody (ET1703-63, green) at 1/100 dilution overnight at 4℃, washed with PBS. Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) was used as the secondary antibody at 1/200 dilution. Nuclei were counterstained with DAPI (blue). Image acquisition was performed with KFBIO KF-FL-400 Scanner.

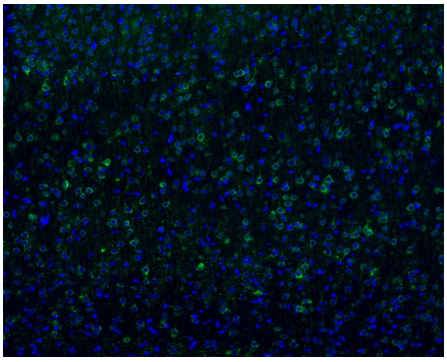

Fig5: Immunofluorescence analysis of frozen mouse cerebral cortex tissue labeling TNF Receptor II with Rabbit anti-TNF Receptor II antibody (ET1703-63).

The tissues were blocked in 3% BSA for 30 minutes at room temperature, washed with PBS, and then probed with the primary antibody (ET1703-63, green) at 1/100 dilution overnight at 4℃, washed with PBS. Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) was used as the secondary antibody at 1/200 dilution. Nuclei were counterstained with DAPI (blue). Image acquisition was performed with KFBIO KF-FL-400 Scanner.

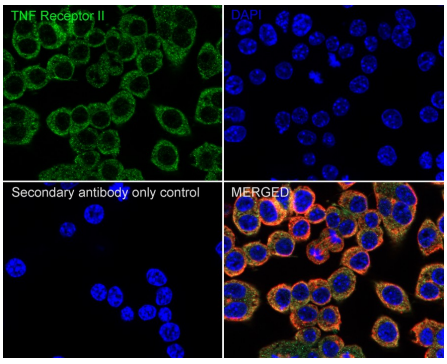

Fig6: Immunocytochemistry analysis of RAW264.7 cells labeling TNF Receptor II with Rabbit anti-TNF Receptor II antibody (ET1703-63) at 1/100 dilution.

Cells were fixed in 4% paraformaldehyde for 20 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 5 minutes at room temperature, then blocked with 1% BSA in 10% negative goat serum for 1 hour at room temperature. Cells were then incubated with Rabbit anti-TNF Receptor II antibody (ET1703-63) at 1/100 dilution in 1% BSA in PBST overnight at 4 ℃. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. PBS instead of the primary antibody was used as the secondary antibody only control. Nuclear DNA was labelled in blue with DAPI.

Beta tubulin (M1305-2, red) was stained at 1/100 dilution overnight at +4℃. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

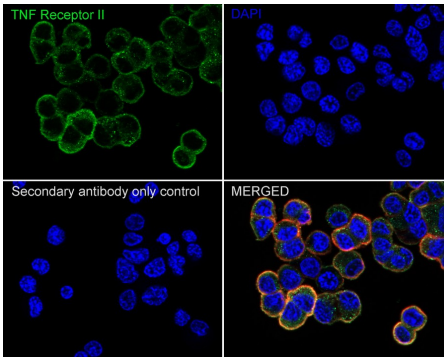

Fig7: Immunocytochemistry analysis of PC-12 cells labeling TNF Receptor II with Rabbit anti-TNF Receptor II antibody (ET1703-63) at 1/100 dilution.

Cells were fixed in 4% paraformaldehyde for 20 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 5 minutes at room temperature, then blocked with 1% BSA in 10% negative goat serum for 1 hour at room temperature. Cells were then incubated with Rabbit anti-TNF Receptor II antibody (ET1703-63) at 1/100 dilution in 1% BSA in PBST overnight at 4 ℃. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. PBS instead of the primary antibody was used as the secondary antibody only control. Nuclear DNA was labelled in blue with DAPI.

Beta tubulin (M1305-2, red) was stained at 1/100 dilution overnight at +4℃. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

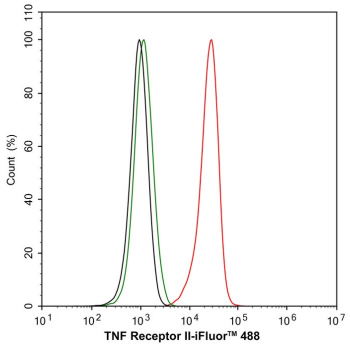

Fig8: Flow cytometric analysis of RAW264.7 cells labeling TNF Receptor II.

Cells were fixed and permeabilized. Then stained with the primary antibody (ET1703-63, 1μg/mL) (red) compared with Rabbit IgG Isotype Control (green). After incubation of the primary antibody at +4℃ for an hour, the cells were stained with a iFluor™ 488 conjugate-Goat anti-Rabbit IgG Secondary antibody (HA1121) at 1/1,000 dilution for 30 minutes at +4℃. Unlabelled sample was used as a control (cells without incubation with primary antibody; black).

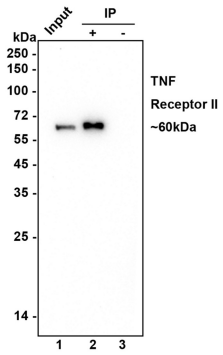

Fig9: TNF Receptor II was immunoprecipitated in 0.2mg Jurkat cell lysate with ET1703-63 at 2 μg/10 μl beads. Western blot was performed from the immunoprecipitate using ET1703-63 at 1/1,000 dilution. Anti-Rabbit IgG for IP Nano-secondary antibody (NBI01H) at 1/5,000 dilution was used for 1 hour at room temperature.

Lane 1: Jurkat cell lysate (input)
Lane 2: ET1703-63 IP in Jurkat cell lysate
Lane 3: Rabbit IgG instead of ET1703-63 in Jurkat cell lysate

Blocking/Dilution buffer: 5% NFDM/TBST
Exposure time: 3 mintues

Background References

1. Gao Y et al. Single-cell transcriptomics identify TNFRSF1B as a novel T-cell exhaustion marker for ovarian cancer. Clin Transl Med. 2023 Sep
2. Carvalho BF et al. TNFRSF1B Gene Variants in Clinicopathological Aspects and Prognosis of Patients with Cutaneous Melanoma. Int J Mol Sci. 2024 Mar