Anti-TDP43 Antibody [JM51-10]

ET1703-74

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat, Zebrafish, Cynomolgus monkey, Pig
Applications:	WB, IP, IF-Cell, IF-Tissue, IHC-P, FC, IHC-Fr
Molecular Wt:	Predicted band size: 45 kDa
Clone number:	JM51-10

Description: TDP-43 is a transcriptional repressor that binds to chromosomally integrated TAR DNA and represses HIV-1 transcription. In addition, this protein regulates alternate splicing of the CFTR gene. TDP-43 has been shown to bind both DNA and RNA and have multiple functions in transcriptional repression, pre-mRNA splicing and translational regulation. Recent work has characterized the transcriptome-wide binding sites revealing that thousands of RNAs are bound by TDP-43 in neurons. TDP-43 was originally identified as a transcriptional repressor that binds to chromosomally integrated trans-activation response element (TAR) DNA and represses HIV-1 transcription. It was also reported to regulate alternate splicing of the CFTR gene and the apoA-II gene. In spinal motor neurons TDP-43 has also been shown in humans to be a low molecular weight neurofilament (hNFL) mRNA-binding protein. It has also shown to be a neuronal activity response factor in the dendrites of hippocampal neurons suggesting possible roles in regulating mRNA stability, transport and local translation in neurons. It has been demonstrated that zinc ions are able to induce aggregation of endogenous TDP-43 in cells. Moreover, zinc could bind to RNA binding domain of TDP-43 and induce the formation of amyloid-like aggregates in vitro.

Immunogen:	Synthetic peptide within Human TDP43 aa 354-390 / 414; (2)aa 253-290 / 414.
Positive control:	HeLa cell lysate, K-562 cell lysate, Neuro-2a cell lysate, mouse spleen tissue lysate, mouse brain tissue lysate, rat spleen tissue lysate, rat brain tissue lysate, HeLa, Neuro-2a, human spleen tissue, mouse placenta tissue, human pancreas tissue, mouse hippocampus tissue, mouse cerebral cortex tissue, mouse cerebrum tissue.
Subcellular location:	Cytoplasm, Mitochondrion, Nucleus.
Database links:	SwissProt: Q13148 Human | Q921F2 Mouse Entrez Gene: 298648 Rat | 325052 Zebrafish

Recommended Dilutions:

WB	1:1,000-1:5,000
IHC-Fr	1:200-1:500
IF-Cell	1:250-1:500
IF-Tissue	1:100
IHC-P	1:500
FC	1:50-1:100
IP	Use at an assay dependent concentration.

Storage Buffer:	1*PBS (pH7.4), 0.1% BSA, 40% Glycerol, 0.2% Proclean 950.
Storage Instruction:	Shipped at 4℃. Store at +4℃ short term (1-2 weeks). Store at -20℃ long term.
Purity:	Protein A affinity purified.

Images

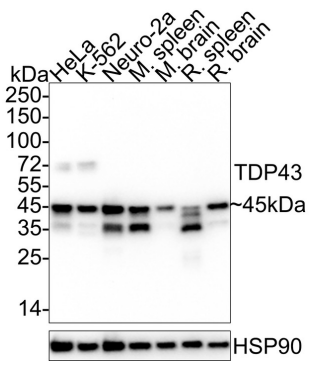

Fig1: Western blot analysis of TDP43 on different lysates with Rabbit anti-TDP43 antibody (ET1703-74) at 1/1,000 dilution.

Lane 1: HeLa cell lysate
Lane 2: K-562 cell lysate
Lane 3: Neuro-2a cell lysate
Lane 4: Mouse spleen tissue lysate
Lane 5: Mouse brain tissue lysate
Lane 6: Rat spleen tissue lysate
Lane 7: Rat brain tissue lysate

Lysates/proteins at 20 µg/Lane.

Predicted band size: 45 kDa
Observed band size: 45 kDa

Exposure time: 20 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 (ET1703-74) at 1/1,000 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.

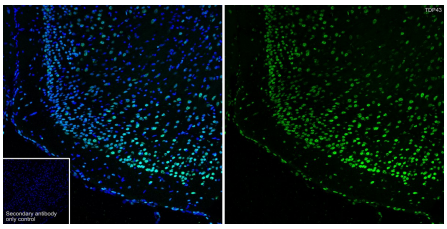

Fig2: Application: IHC-Fr

Species: Mouse

Site: Cerebral cortex

Sample: Frozen section

Antibody concentration: 1/200

Antigen retrieval: The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) for about 2 minutes in microwave oven.

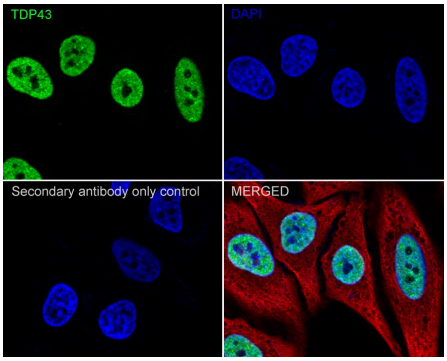

Fig3: Immunocytochemistry analysis of HeLa cells labeling TDP43 with Rabbit anti-TDP43 antibody (ET1703-74) at 1/250 dilution.

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-TDP43 antibody (ET1703-74) at 1/250 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.

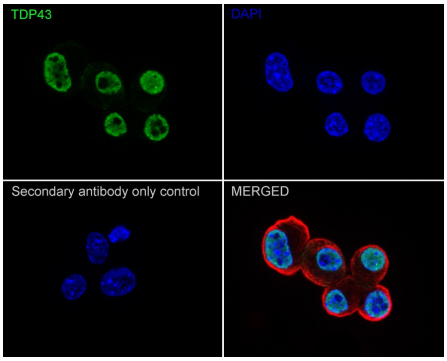

Fig4: Immunocytochemistry analysis of Neuro-2a cells labeling TDP43 with Rabbit anti-TDP43 antibody (ET1703-74) at 1/500 dilution.

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-TDP43 antibody (ET1703-74) at 1/500 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.

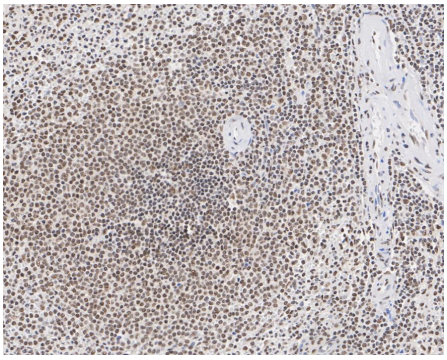

Fig5: Immunohistochemical analysis of paraffin-embedded human spleen tissue with Rabbit anti-TDP43 antibody (ET1703-74) at 1/1,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) (high pressure) for 2 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1703-74) at 1/1,000 dilution for 1 hour 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.

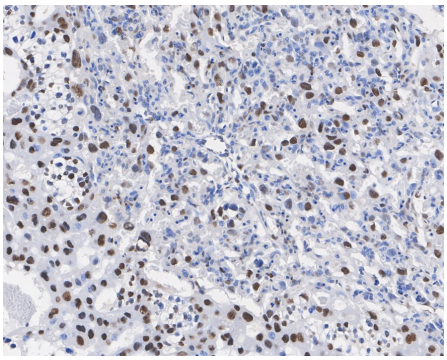

Fig6: Immunohistochemical analysis of paraffin-embedded mouse placenta tissue with Rabbit anti-TDP43 antibody (ET1703-74) at 1/1,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) (high pressure) for 2 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1703-74) at 1/1,000 dilution for 1 hour 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.

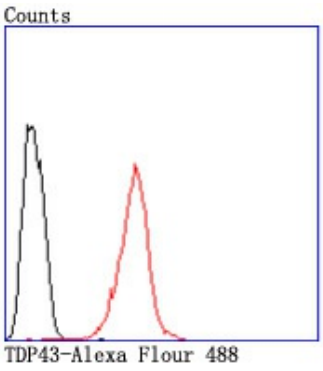

Fig7: Flow cytometric analysis of TDP43 was done on Hela cells. The cells were fixed, permeabilized and stained with the primary antibody (ET1703-74, 1/50) (red). After incubation of the primary antibody at room temperature for an hour, the cells were stained with a Alexa Fluor 488-conjugated Goat anti-Rabbit IgG Secondary antibody at 1/1000 dilution for 30 minutes.Unlabelled sample was used as a control (cells without incubation with primary antibody; black).

Note: All products are "FOR RESEARCH USE ONLY AND ARE NOT INTENDED FOR DIAGNOSTIC OR THERAPEUTIC USE".

Background References

1. McMillan M et al. RNA methylation influences TDP43 binding and disease pathogenesis in models of amyotrophic lateral sclerosis and frontotemporal dementia. Mol Cell. 2023 Jan
2. Corbet GA et al. TDP43 ribonucleoprotein granules: physiologic function to pathologic aggregates. RNA Biol. 2021 Oct

Applications:WB=Western blot IHC-P=Immunohistochemistry (paraffin) IF-Cell=Immunofluorescence (Cell) IF-Tissue=Immunofluorescence (Tissue) FC=Flow cytometry IP=Immunoprecipitation