abcam

Product datasheet

Anti-TDP43 antibody [EPR18554] ab190963

KO VALIDATED Recombinant RabMAb

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Overview

Product name	Anti-TDP43 antibody [EPR18554]
Description	Rabbit monoclonal [EPR18554] to TDP43
Host species	Rabbit
Tested applications	**Suitable for:** WB, IHC-P, ICC/IF, IP, Flow Cyt (Intra)
Species reactivity	**Reacts with:** Human, Zebrafish **Predicted to work with:** Mouse, Rat
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: HeLa, HEK-293 and K562 whole cell lysates; Human fetal brain and fetal heart lysates; Zebrafish head lysate; Untreated HeLa whole cell lysates and HeLa whole cell lysates treated with 1ÂµM staurosporine for 4 hours, 50 ÂµM Z-VAD-FMK for 4 hours and 1ÂµM staurosporine and 50 ÂµM Z-VAD-FMK for 4 hours. IHC-P: Human pancreas and endometrium carcinoma tissues; Mouse and rat liver tissues. ICC/IF: HeLa and K562 cells. Flow Cyt (intra): K562 cells. IP: HeLa whole cell lysate.
General notes	This product is a recombinant monoclonal antibody, which offers several advantages including: - High batch-to-batch consistency and reproducibility - Improved sensitivity and specificity - Long-term security of supply - Animal-free production For more information **see here**. Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to **RabMAb® patents**.

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C long term. Avoid freeze / thaw cycle.
Storage buffer	pH: 7.2 Preservative: 0.01% Sodium azide Constituents: PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA

Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR18554
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab190963 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

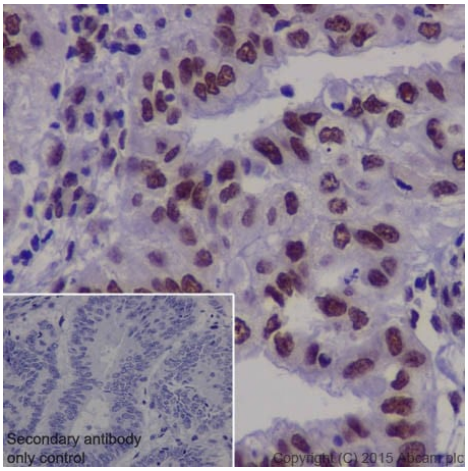
Application	Abreviews	Notes
WB	★★★★☆ (1)	1/1000. Detects a band of approximately 45 kDa (predicted molecular weight: 45 kDa).
IHC-P		1/1000. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
ICC/IF		1/1000.
IP		1/40.
Flow Cyt (Intra)		1/1000.

Target

Function	DNA and RNA-binding protein which regulates transcription and splicing. Involved in the regulation of CFTR splicing. It promotes CFTR exon 9 skipping by binding to the UG repeated motifs in the polymorphic region near the 3'-splice site of this exon. The resulting aberrant splicing is associated with pathological features typical of cystic fibrosis. May also be involved in microRNA biogenesis, apoptosis and cell division. Can repress HIV-1 transcription by binding to the HIV-1 long terminal repeat. Stabilizes the low molecular weight neurofilament (NFL) mRNA through a direct interaction with the 3' UTR.
Tissue specificity	Ubiquitously expressed. In particular, expression is high in pancreas, placenta, lung, genital tract and spleen.
Involvement in disease	Defects in TARDBP are the cause of amyotrophic lateral sclerosis type 10 (ALS10) [MIM:612069]. ALS is a neurodegenerative disorder affecting upper and lower motor neurons and resulting in fatal paralysis. Sensory abnormalities are absent. Death usually occurs within 2 to 5 years. The etiology of ALS is likely to be multifactorial, involving both genetic and environmental factors. The disease is inherited in 5-10% of the cases.
Sequence similarities	Contains 2 RRM (RNA recognition motif) domains.
Domain	The RRM domains can bind to both DNA and RNA.
Post-translational modifications	Hyperphosphorylated in hippocampus, neocortex, and spinal cord from individuals affected with ALS and FTLDU. Ubiquitinated in hippocampus, neocortex, and spinal cord from individuals affected with ALS and FTLDU. Cleaved to generate C-terminal fragments in hippocampus, neocortex, and spinal cord from

	individuals affected with ALS and FTLDU.
Cellular localization	Nucleus. In patients with frontotemporal lobar degeneration and amyotrophic lateral sclerosis, it is absent from the nucleus of affected neurons but it is the primary component of cytoplasmic ubiquitin-positive inclusion bodies.

Images



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-TDP43 antibody [EPR18554] (ab190963)

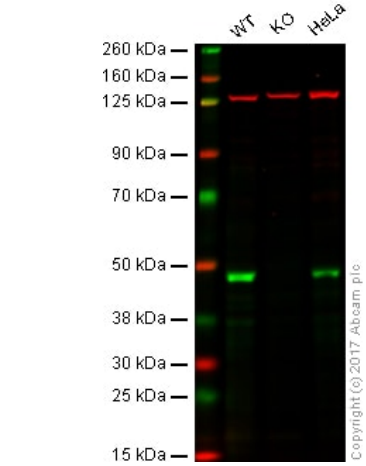
Immunohistochemical analysis of paraffin-embedded Human endometrium carcinoma tissue labeling TDP43 with ab190963 at 1/1000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/500 dilution.

Nucleus staining on tumor cells of the endometrium carcinoma is observed.

Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is **ab97051** at 1/500 dilution.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

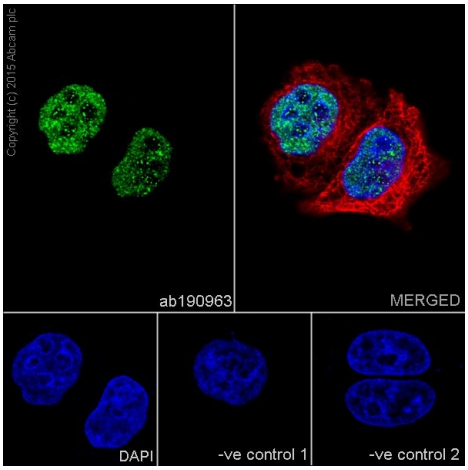


Western blot - Anti-TDP43 antibody [EPR18554] (ab190963)

Lane 1: Wild type HAP1 whole cell lysate (20 µg)
Lane 2: TDP43 knockout HAP1 whole cell lysate (20 µg)
Lane 3: HeLa whole cell lysate (20 µg)

Lanes 1 - 3: Merged signal (red and green). Green - ab190963 observed at 48 kDa. Red - loading control, **ab18058**, observed at 130 kDa.

ab190963 was shown to specifically react with TDP43 in wild type cells as signal was lost in TDP43 knockout cells. Wild-type and TDP43 knockout samples were subjected to SDS-PAGE. ab190963 and **ab18058** (Mouse anti-Vinculin loading control) were incubated overnight at 4°C at 1/1000 dilution and 1/20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed **ab216773** and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed **ab216776** secondary antibodies at 1/20000 dilution for 1 hour at room temperature before imaging.



Immunocytochemistry/ Immunofluorescence - Anti-TDP43 antibody [EPR18554] (ab190963)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized HeLa (Human epithelial cell line from cervix adenocarcinoma) cells labeling TDP43 with ab190963 at 1/1000 dilution, followed by Goat Anti-Rabbit IgG (Alexa Fluor® 488) (**ab150077**) secondary antibody at 1/1000 dilution (green).

Confocal image showing nuclear staining on HeLa cell line.

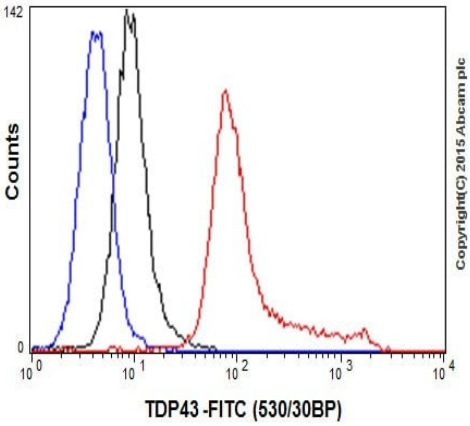
The nuclear counterstain is DAPI (blue).

Tubulin is detected with Anti-alpha Tubulin antibody [DM1A] - Loading Control (**ab7291**) at 1/1000 dilution and Goat Anti-Mouse IgG H&L (Alexa Fluor® 594) preadsorbed (**ab150120**) at 1/1000 dilution (red).

The negative controls are as follows:-

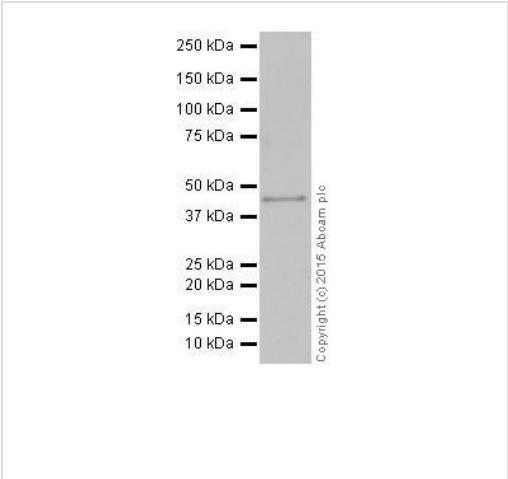
-ve control 1: ab190963 at 1/2000 dilution followed by **ab150120** at 1/1000 dilution.

-ve control 2: **ab7291** at 1/1000 dilution followed by **ab150077** at 1/1000 dilution.



Flow Cytometry (Intracellular) - Anti-TDP43 antibody [EPR18554] (ab190963)

Intracellular flow cytometric analysis of 4% paraformaldehyde-fixed K562 (Human chronic myelogenous leukemia cell line from bone marrow) cells labeling TDP43with ab190963 at 1/1000 dilution (red) compared with a Rabbit IgG,monoclonal [EPR25A] -Isotype control (**ab172730**) (black) and an unlabelled control (cells without incubation with primary antibody and secondary antibody) (blue). Goat anti Rabbit IgG (FITC) at 1/500 dilution was used as the secondary antibody.



Western blot - Anti-TDP43 antibody [EPR18554] (ab190963)

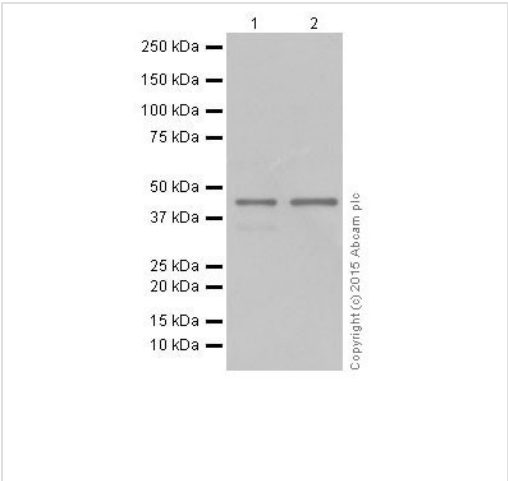
Anti-TDP43 antibody [EPR18554] (ab190963) at 1/2000 dilution + HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate at 20 µg

Secondary
Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/100000 dilution

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

Exposure time: 3 seconds

Blocking/Dilution buffer: 5% NFDM/TBST.



Western blot - Anti-TDP43 antibody [EPR18554] (ab190963)

All lanes : Anti-TDP43 antibody [EPR18554] (ab190963) at 1/10000 dilution

Lane 1 : HEK-293 (Human epithelial cell line from embryonic kidney) whole cell lysate lysate
Lane 2 : K562 (Human chronic myelogenous leukemia cell line from bone marrow) whole cell lysate lysate

Lysates/proteins at 20 µg per lane.

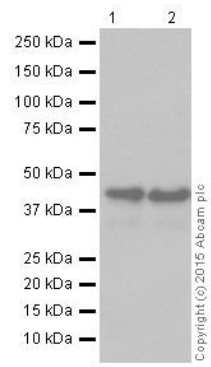
Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/100000 dilution

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

Exposure time: 5 seconds

Blocking/Dilution buffer: 5% NFDM/TBST.

The band of ~35 kDa is the cleavage product from caspase activity (PMID:22659571, PMID:20736350).



Western blot - Anti-TDP43 antibody [EPR18554] (ab190963)

All lanes : Anti-TDP43 antibody [EPR18554] (ab190963) at 1/1000 dilution

Lane 1 : Human fetal brain lysate
Lane 2 : Human fetal heart lysate

Lysates/proteins at 10 µg per lane.

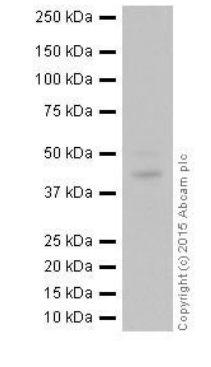
Secondary
All lanes : Anti-Rabbit IgG (HRP), specific to the non-reduced form of IgG at 1/10000 dilution

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

Exposure time: 10 seconds

Blocking/Dilution buffer: 5% NFDM/TBST.

The band of ~35 kDa is the cleavage product from caspase activity (PMID:22659571, PMID:20736350).



Western blot - Anti-TDP43 antibody [EPR18554] (ab190963)

Anti-TDP43 antibody [EPR18554] (ab190963) at 1/1000 dilution + Zebrafish head lysate at 10 µg

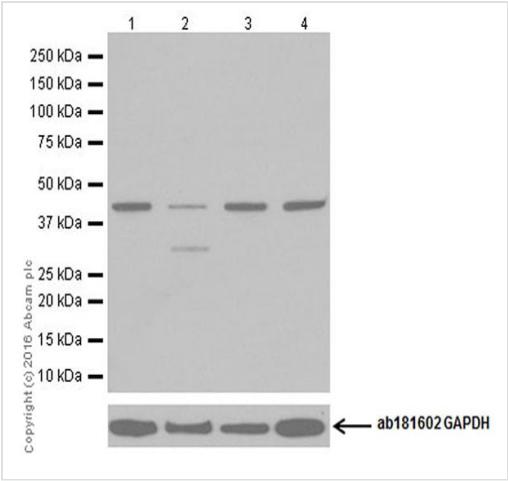
Secondary
Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/100000 dilution

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

Exposure time: 3 minutes

Blocking/Dilution buffer: 5% NFDM/TBST.

The band of ~35 kDa is the cleavage product from caspase activity (PMID:22659571, PMID:20736350).



Western blot - Anti-TDP43 antibody [EPR18554] (ab190963)

All lanes : Anti-TDP43 antibody [EPR18554] (ab190963) at 1/1000 dilution

Lane 1 : Untreated HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate
Lane 2 : HeLa (Human epithelial cell line from cervix adenocarcinoma) treated with 1µM staurosporine for 4 hours whole cell lysate
Lane 3 : HeLa (Human epithelial cell line from cervix adenocarcinoma) treated with 50 µM Z-VAD-FMK for 4 hours whole cell lysate
Lane 4 : HeLa (Human epithelial cell line from cervix adenocarcinoma) treated with 1µM staurosporine and 50 µM Z-VAD-FMK for 4 hours whole cell lysate

Lysates/proteins at 20 µg per lane.

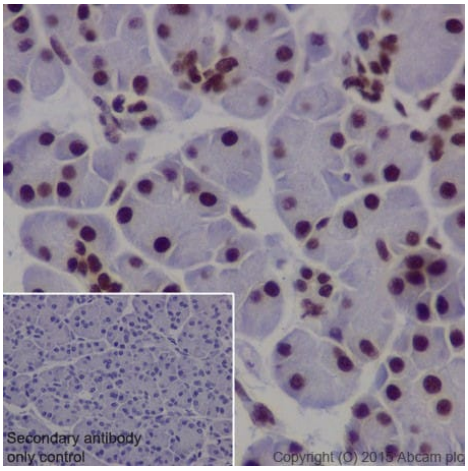
Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/100000 dilution

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

Exposure time: 3 minutes

Blocking/Dilution buffer: 5% NFDM/TBST.

The band of ~35 kDa is the cleavage product from caspase activity (PMID:22659571, PMID:20736350), and can be induced by staurosporine.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-TDP43 antibody [EPR18554] (ab190963)

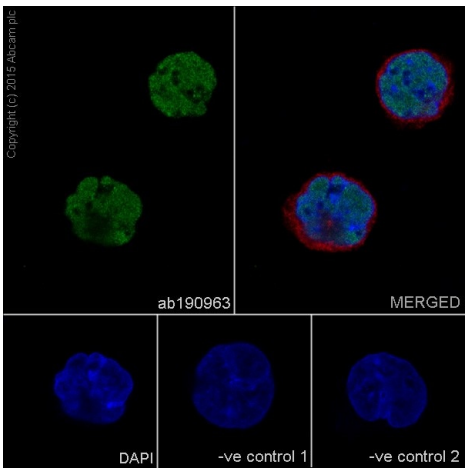
Immunohistochemical analysis of paraffin-embedded Human pancreas tissue labeling TDP43 with ab190963 at 1/1000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/500 dilution.

Nucleus staining on normal Human pancreas is observed.

Counter stained with Hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is **ab97051** at 1/500 dilution.

Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.



Immunocytochemistry/ Immunofluorescence - Anti-TDP43 antibody [EPR18554] (ab190963)

Immunofluorescent analysis of 4% paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized K562 (Human chronic myelogenous leukemia cell line from bone marrow) cells labeling TDP43 with ab190963 at 1/1000 dilution, followed by Goat Anti-Rabbit IgG (Alexa Fluor® 488) (**ab150077**) secondary antibody at 1/1000 dilution (green).

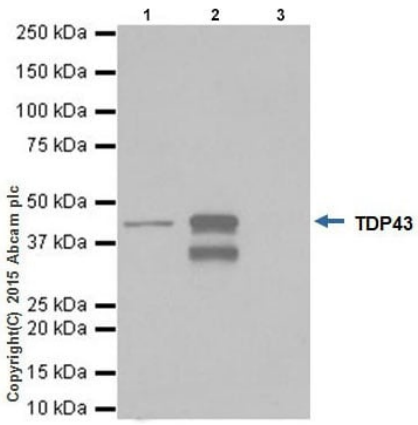
Confocal image showing nuclear staining on K562 cell line.

Tubulin is detected with Anti-alpha Tubulin antibody [DM1A] - Loading Control (**ab7291**) at 1/1000 dilution and Goat Anti-Mouse IgG H&L (Alexa Fluor® 594) preadsorbed (**ab150120**) at 1/1000 dilution (red).

The negative controls are as follows:-

-ve control 1: ab190963 at 1/2000 dilution followed by **ab150120** at 1/1000 dilution.

-ve control 2: **ab7291** at 1/1000 dilution followed by **ab150077** at 1/1000 dilution.



Immunoprecipitation - Anti-TDP43 antibody [EPR18554] (ab190963)

TDP43 was immunoprecipitated from 1mg of HeLa (Human epithelial cell line from cervix adenocarcinoma) whole cell lysate with ab190963 at 1/40 dilution.

Western blot was performed from the immunoprecipitate using ab190963 at 1/1000 dilution.

VeriBlot for IP Detection Reagent (HRP) (**ab131366**), was used for detection at 1/10000 dilution.

Lane 1: HeLa whole cell lysate, 10µg (Input).

Lane 2: ab190963 IP in HeLa whole cell lysate.

Lane 3: Rabbit IgG,monoclonal [EPR25A] - Isotype Control (**ab172730**) instead of ab190963 in HeLa whole cell lysate.

Blocking and dilution buffer and concentration: 5% NFDM/TBST.

Exposure time: 1 second.

The band of ~35 kDa is the cleavage product from caspase activity (PMID:22659571, PMID:20736350).

Anti-TDP43 antibody [EPR18554] (ab190963)

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