abcam

Product datasheet

Anti-TDP43 antibody [EPR5810] ab109535

KO VALIDATED Recombinant RabMAb

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Overview

Product name	Anti-TDP43 antibody [EPR5810]
Description	Rabbit monoclonal [EPR5810] to TDP43
Host species	Rabbit
Tested applications	**Suitable for:** ICC/IF, IHC-Fr, WB, IHC-P, Flow Cyt (Intra) **Unsuitable for:** IP
Species reactivity	**Reacts with:** Mouse, Rat, Human
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: HAP1, HeLa, Jurkat, 293T, K562, and A431 cell lysates, Mouse and Rat brain lysates; IHC-Fr: Mouse cerebrum tissue, Human prostate carcinoma IHC-P: Human papillary carcinoma and glioma tissue, Mouse and Rat cerebrum tissues; ICC/IF: HAP1-TARDBP, Hek293 and HeLa cells; Flow Cyt (intra): K562 cells.
General notes	TARDBP is a protein encoded by the TARDBP gene. A hyper-phosphorylated, ubiquitinated and cleaved formof TARDBP, known as TDP-43 is the significant protein in several diseases, including amyotrophic lateral sclerosis (ALS). 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**. **We are constantly working hard to ensure we provide our customers with best in class antibodies. As a result of this work we are pleased to now offer this antibody in purified format. We are in the process of updating our datasheets. The purified format is designated 'PUR' on our product labels. If you have any questions regarding this update, please contact our Scientific Support team.**

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Upon delivery aliquot and store at -20°C. Avoid freeze / thaw cycles.
Storage buffer	pH: 7.20 Preservative: 0.01% Sodium azide Constituents: PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR5810
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab109535 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
ICC/IF		Use a concentration of 1 µg/ml. This antibody is suitable to detect TDP43 using MeOH fixation in ICC. We have compared methanol and paraformaldehyde (PFA) fixation methods with this product and recommend to use methanol only.
IHC-Fr		1/50. Heat mediated antigen retrieval using sodium citrate buffer (10mM citrate pH 6.0 + 0.05% Tween-20)
WB		1/1000 - 1/10000. Predicted molecular weight: 45 kDa.
IHC-P		1/100 - 1/250. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.
Flow Cyt (Intra)		Use at an assay dependent concentration.

Application notes Is unsuitable for IP.

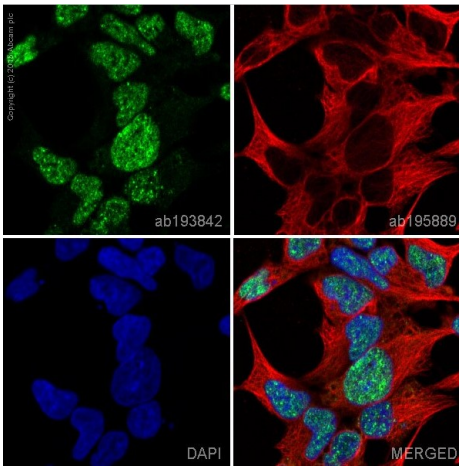
Target

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

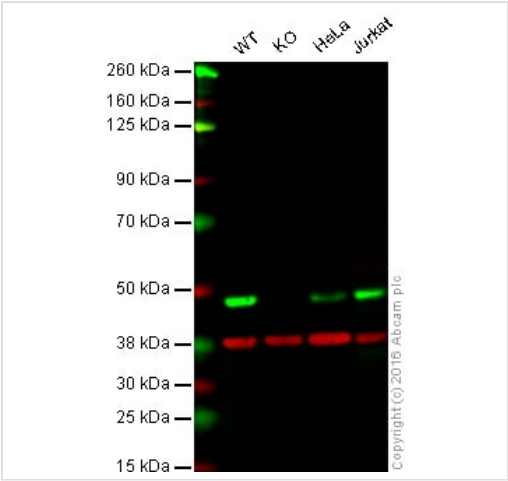


Immunocytochemistry/ Immunofluorescence - Anti-TDP43 antibody [EPR5810] (ab109535)

ab193842 staining TDP43 in Hek293 cells. The cells were fixed with 100% methanol (5 min), permeabilized with 0.1% Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1%PBS-Tween for 1h. The cells were then incubated overnight at +4°C with **ab193842** at a 1/250 dilution (shown in green) and **ab195889**, Mouse monoclonal to alpha Tubulin (Alexa Fluor® 594), at a 1/250 dilution (shown in red). Nuclear DNA was labelled with DAPI (shown in blue).

Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).

This antibody is not suitable to detect TDP43 using PFA fixation in ICC.

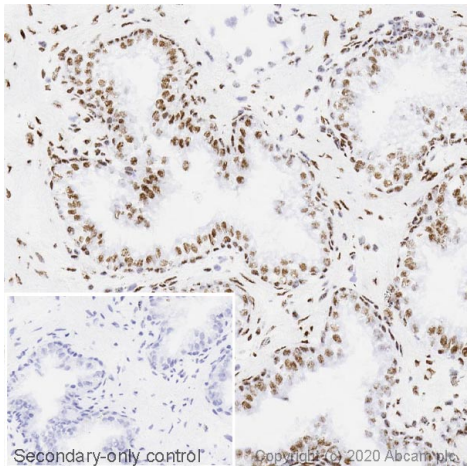


Western blot - Anti-TDP43 antibody [EPR5810] (ab109535)

Lane 1: Wild-type HAP1 cell lysate (40 µg)
Lane 2: TDP43 knockout HAP1 cell lysate (40 µg)
Lane 3: HeLa cell lysate (40 µg)
Lane 4: Jurkat cell lysate (40 µg)

Lanes 1 - 4: Merged signal (red and green). Green - ab109535 observed at 48 kDa. Red - loading control, **ab8245**, observed at 37 kDa.

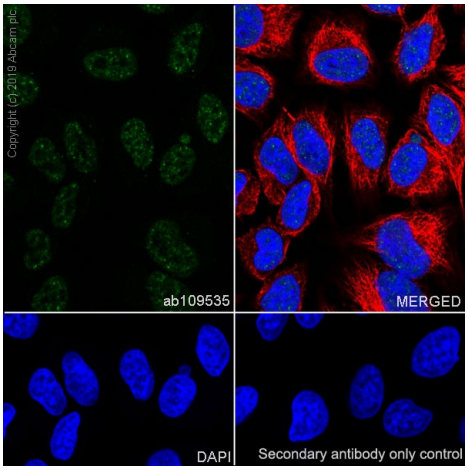
Unpurified ab109535 was shown to specifically react with TDP43 when TDP43 knockout samples were used. Wild-type and TDP43 knockout samples were subjected to SDS-PAGE. Ab109535 and **ab8245** (loading control to GAPDH) were diluted at 1/1000 and 1/10,000 dilution respectively and incubated overnight at 4C. Blots were developed with IRDye® 800CW Goat anti-Rabbit IgG (H + L) and IRDye® 680 Goat anti-Mouse IgG (H + L) secondary antibodies at 1/10,000 dilution for 1 hour at room temperature before imaging.



Immunohistochemistry (Frozen sections) - Anti-TDP43 antibody [EPR5810] (ab109535)

IHC image of TDP43 staining in a section of frozen human prostate carcinoma performed on a Leica BOND™ system using the standard protocol. The section was fixed in 10% paraformaldehyde (10 min) prior to staining. The section was incubated with ab109535, 1/100 dilution, for 15 mins at room temperature and detected using an HRP conjugated compact polymer system. DAB was used as the chromogen. The section was then counterstained with haematoxylin and mounted with DPX. The inset secondary-only control image is taken from an identical assay without primary antibody.

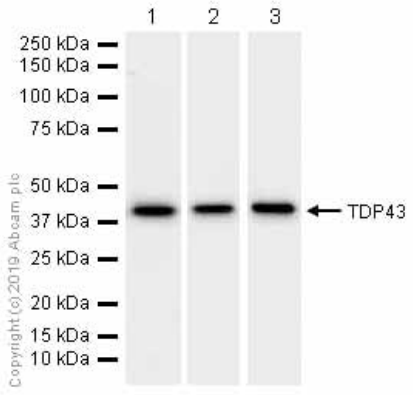
For other IHC staining systems (automated and non-automated) customers should optimize variable parameters such as antigen retrieval conditions, primary antibody concentration and antibody incubation times.



Immunocytochemistry/ Immunofluorescence - Anti-TDP43 antibody [EPR5810] (ab109535)

Immunocytochemistry/ Immunofluorescence analysis of HeLa (Human cervix adenocarcinoma epithelial cell) cells labeling TDP43 with purified ab109535 at 1/50 dilution (6.2 µg/ml). Cells were fixed in 100% Methanol and permeabilized with None. Cells were counterstained with Ab195889 Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) 1/200 (2.5 µg/ml). Goat anti rabbit IgG (Alexa Fluor® 488, **ab150077**) was used as the secondary antibody at 1/1000 (2 µg/ml) dilution. DAPI (blue) was used as nuclear counterstain. PBS instead of the primary antibody was used as the secondary antibody only control.

This antibody is not suitable to detect TDP43 using PFA fixation in ICC.



Western blot - Anti-TDP43 antibody [EPR5810] (ab109535)

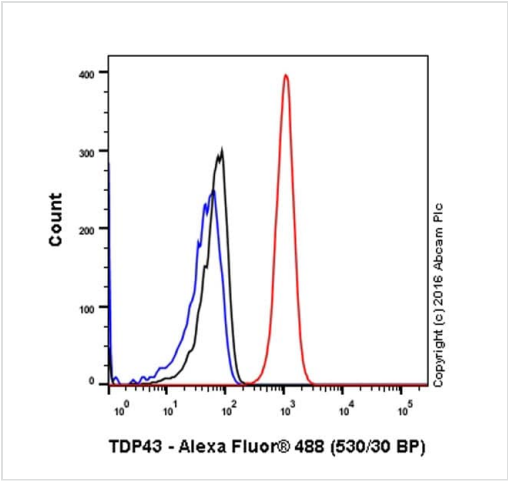
All lanes : Anti-TDP43 antibody [EPR5810] (ab109535) at 1/5000 dilution (Purified)

Lane 1 : HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysates
Lane 2 : Mouse brain lysates
Lane 3 : Rat brain lysates

Lysates/proteins at 15 µg per lane.

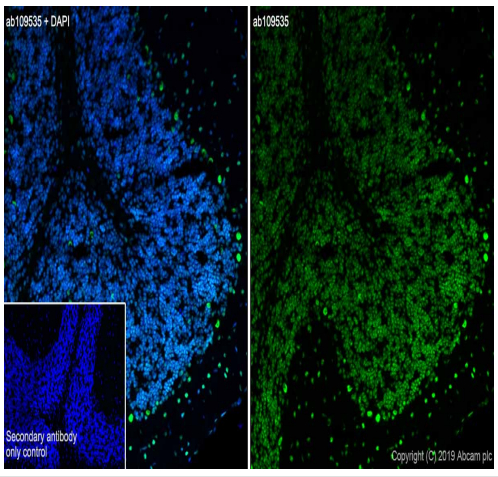
Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/20000 dilution

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



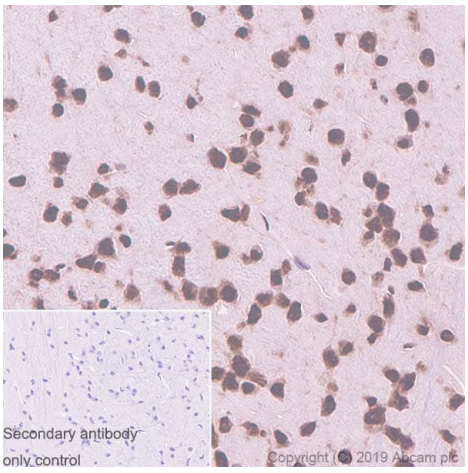
Flow Cytometry (Intracellular) - Anti-TDP43 antibody [EPR5810] (ab109535)

Intracellular Flow Cytometry analysis of K562 (human chronic myelogenous leukemia) cells labeling TDP43 with purified ab109535 at 1/20 dilution (10ug/ml) (red). Cells were fixed with 4% paraformaldehyde and permeabilised with 90% methanol. A Goat anti rabbit IgG (Alexa Fluor® 488) (1/2000 dilution) was used as the secondary antibody. Rabbit monoclonal IgG (Black) was used as the isotype control, cells without incubation with primary antibody and secondary antibody (Blue) were used as the unlabeled control.



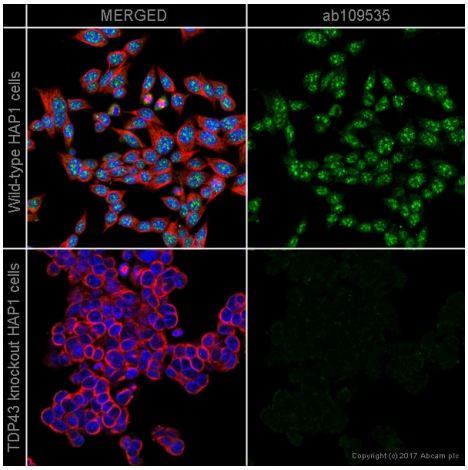
Immunohistochemistry (Frozen sections) - Anti-TDP43 antibody [EPR5810] (ab109535)

Immunohistochemistry (Frozen sections) analysis of mouse cerebrum tissue sections labeling TDP43 with Purified unpurified ab109535 at 1/50 (0.5 µg/ml).Heat mediated antigen retrieval using sodium citrate buffer (10mM citrate pH 6.0 + 0.05% Tween-20). Goat anti rabbit IgG (Alexa Fluor® 488, **ab150077**) was used as the secondary antibody. Negative control: PBS instead of the primary antibody. DAPI was used as a counterstain.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-TDP43 antibody [EPR5810] (ab109535)

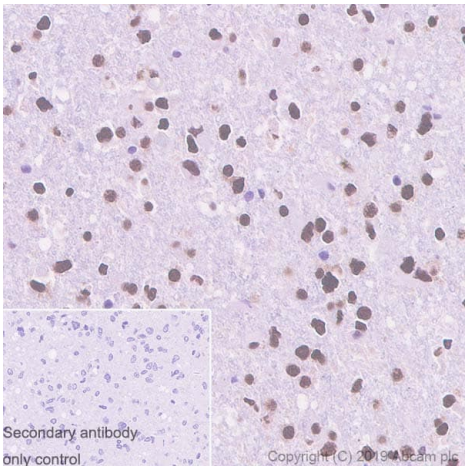
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of mouse cerebrum tissue sections labeling TDP43 with purified ab109535 at 1/100 dilution (0.3 µg/ml). Heat mediated antigen retrieval using Bond™ Epitope Retrieval Solution 2 (pH 9.0). Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**) was used as the secondary antibody. Negative control: PBS instead of the primary antibody. Hematoxylin was used as a counterstain.



Immunocytochemistry/ Immunofluorescence - Anti-TDP43 antibody [EPR5810] (ab109535)

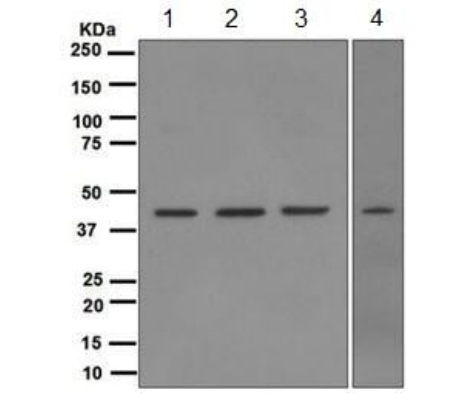
Unpurified ab109535 staining TDP43 in wild-type HAP1 cells (top panel) and TDP43 knockout HAP1 cells (bottom panel). The cells were fixed with 100% methanol (5min), permeabilized with 0.1% Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated with ab109535 at 1µg/ml concentration and **ab195889** at 1/250 dilution (shown in pseudo colour red) overnight at +4°C, followed by a further incubation at room temperature for 1h with a goat secondary antibody to Rabbit IgG (Alexa Fluor® 488) (**ab150081**) at 2 µg/ml (shown in green). Nuclear DNA was labelled in blue with DAPI.

This antibody is not suitable to detect TDP43 using PFA fixation in ICC.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-TDP43 antibody [EPR5810] (ab109535)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of human glioma tissue sections labeling TDP43 with purified ab109535 at 1/100 dilution (0.3 µg/ml). Heat mediated antigen retrieval using Bond™ Epitope Retrieval Solution 2 (pH 9.0). Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**) was used as the secondary antibody. Negative control: PBS instead of the primary antibody. Hematoxylin was used as a counterstain.



Western blot - Anti-TDP43 antibody [EPR5810] (ab109535)

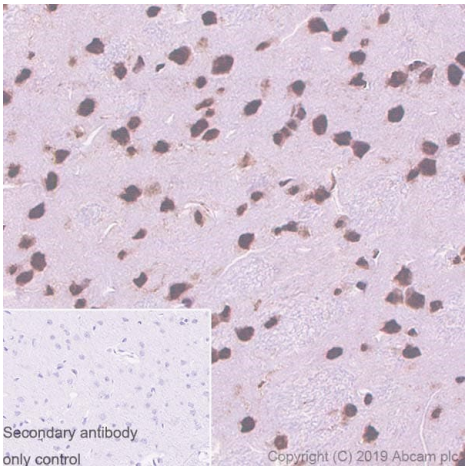
All lanes : Anti-TDP43 antibody [EPR5810] (ab109535) at 1/1000 dilution ((unpurified))

Lane 1 : HeLa cell lysate
Lane 2 : 293T cell lysate
Lane 3 : K562 cell lysate
Lane 4 : A431 cell lysate

Lysates/proteins at 10 µg per lane.

Secondary
All lanes : HRP-labelled goat anti-rabbit at 1/2000 dilution

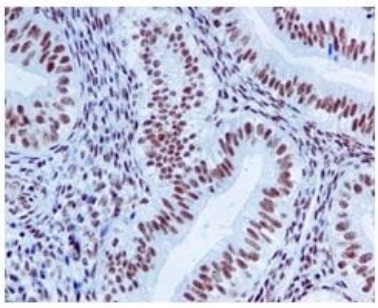
Predicted band size: 45 kDa



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-TDP43 antibody [EPR5810] (ab109535)

Immunohistochemical analysis of paraffin-embedded Rat cerebrum tissue labeling TDP43 with unpurified ab109535, followed by a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**). Nuclear staining on rat cerebrum.The section was incubated with **ab229902** for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Counterstained with Hematoxylin. Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution 2)

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (**ab209101**).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-TDP43 antibody [EPR5810] (ab109535)

Unpurified ab109535 at 1/100 dilution staining TARDBP in paraffin-embedded Human papillary carcinoma tissue by Immunohistochemistry.

Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.

Anti-TDP43 antibody [EPR5810] (ab109535)

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