abcam

ab180191

Anti-USP9x antibody [EPR13809(B)] - N-terminal

Recombinant | RabMAb | KO Validated

Key facts

Isotype	IgG
Host species	Rabbit
Storage buffer	pH: 7.2 - 7.4 Preservative: 0.01% Sodium azide Constituents: PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
Form	Liquid
Clonality	Monoclonal
Immunogen	The exact immunogen used to generate this antibody is proprietary information.
Clone number	EPR13809(B)
Purification technique	Affinity purification Protein A
Concentration	0.126 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

IP

Tested

Species	Mouse
Dilution info	1/20
Notes	-

Species	Human

Dilution info	1/20
Notes	-

Predicted

Species	Rat
Dilution info	-
Notes	-

WB

Tested

Species	Mouse
Dilution info	1/1000 - 1/10000
Notes	-

Species	Human
Dilution info	1/1000 - 1/10000
Notes	-

Predicted

Species	Rat
Dilution info	-
Notes	-

ICC/IF

Tested

Species	Human
Dilution info	1/100 - 1/250
Notes	-

Expected

Species	Mouse
Dilution info	Use at an assay dependent concentration.
Notes	-

Predicted

Species	Rat
Dilution info	-
Notes	-

Flow Cyt (Intra)

Tested

Species	Human
Dilution info	1/10 - 1/100
Notes	ab172730 - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.

Expected

Species	Mouse
Dilution info	Use at an assay dependent concentration.
Notes	-

Predicted

Species	Rat
Dilution info	-
Notes	-

IHC-P

Tested

Species	Human

Dilution info	1/50 - 1/100
Notes	Antigen retrieval is recommended. Perform heat-mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

Expected

Species	Mouse
Dilution info	Use at an assay dependent concentration.
Notes	-

Predicted

Species	Rat
Dilution info	-
Notes	-

Target data

See full target information USP9X

Function

Deubiquitinase involved both in the processing of ubiquitin precursors and of ubiquitinated proteins (PubMed:18254724, PubMed:19135894, PubMed:22371489, PubMed:25944111, PubMed:29626158, PubMed:30914461, PubMed:37454738). May therefore play an important regulatory role at the level of protein turnover by preventing degradation of proteins through the removal of conjugated ubiquitin (PubMed:18254724, PubMed:19135894, PubMed:22371489, PubMed:25944111, PubMed:29626158, PubMed:30914461, PubMed:37454738). Specifically hydrolyzes 'Lys-11'-, followed by 'Lys-63'-, 'Lys-48'- and 'Lys-6'-linked polyubiquitins chains (PubMed:30914461). Essential component of TGF-beta/BMP signaling cascade (PubMed:19135894). Specifically deubiquitinates monoubiquitinated SMAD4, opposing the activity of E3 ubiquitin-protein ligase TRIM33 (PubMed:19135894). Deubiquitinates alkylation repair enzyme ALKBH3 (PubMed:25944111). OTUD4 recruits USP7 and USP9X to stabilize ALKBH3, thereby promoting the repair of alkylated DNA lesions (PubMed:25944111). Deubiquitinates RNA demethylase enzyme ALKBH5, promoting its stability (PubMed:37454738). Deubiquitinates mTORC2 complex component RICTOR at 'Lys-294' by removing 'Lys-63'-linked polyubiquitin chains, stabilizing RICTOR and enhancing its binding to MTOR, thus promoting mTORC2 complex assembly (PubMed:33378666). Regulates chromosome alignment and segregation in mitosis by regulating the localization of BIRC5/survivin to mitotic centromeres (PubMed:16322459). Involved in axonal growth and neuronal cell migration (PubMed:24607389). Regulates cellular clock function by enhancing the protein stability and transcriptional activity of the core circadian protein BMAL1 via its deubiquitinating activity (PubMed:29626158). Acts

as a regulator of peroxisome import by mediating deubiquitination of PEX5: specifically deubiquitinates PEX5 monoubiquitinated at 'Cys-11' following its retrotranslocation into the cytosol, resetting PEX5 for a subsequent import cycle (PubMed:22371489). Deubiquitinates PEG10 (By similarity). Inhibits the activation of the Hippo signaling pathway via deubiquitination of AMOTL2 at 'Lys-347' and 'Lys-408' which prohibits its interaction with and activation of LATS2. Loss of LATS2 activation and subsequent loss of YAP1 phosphorylation results in an increase in YAP1-driven transcription of target genes (PubMed:26598551, PubMed:34404733).

Storage

Shipped at conditions	Conditional Ambient
Appropriate short-term storage duration	1-2 weeks
Appropriate short-term storage conditions	+4°C
Appropriate long-term storage conditions	-20°C
Aliquoting information	Upon delivery aliquot
Storage information	Avoid freeze / thaw cycle

Notes

Patented technology
Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAb® patents.

What are the advantages of a recombinant monoclonal antibody?
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 batch production

For more information, read more on recombinant antibodies.

Supplementary info

This supplementary information is collated from multiple sources and compiled automatically.

Activity summary	USP9x also known as ubiquitin-specific peptidase 9 X-linked is a deubiquitinating enzyme that plays a critical role in protein homeostasis. USP9x belongs to the USP

	family and has an approximate molecular mass of 290 kDa. It is expressed broadly in human tissues with particularly high levels in the brain and pancreas. Mechanically USP9x functions to remove ubiquitin molecules from ubiquitinated substrates preventing their degradation by the proteasome. This regulatory action influences many cellular processes by stabilizing specific proteins.
Biological function summary	USP9x influences numerous cellular mechanisms by maintaining protein balance. It is a part of the complex cellular machinery involved in signal transduction cell division and apoptosis. While its enzyme activity regulates various cellular proteins USP9x is essential in neural development and differentiation. The proteolytic activity helps to modulate pathways by controlling protein turnover which impacts cellular responses and development. Failure to regulate proteins accurately can lead to compromised cellular functions.
Pathways	USP9x participates prominently in the Wnt signaling and TGF-beta pathways. Its role in deubiquitination allows it to stabilize proteins like beta-catenin and SMAD4 which are vital for transmitting signals within these pathways. Through these interactions USP9x aids in cell proliferation migration and differentiation often connecting with other proteins like APC or Axin in the Wnt pathway. This demonstrates how USP9x's enzymatic activity intricately links to key biological signaling mechanisms.
Associated diseases and disorders	Mutations and dysregulation of USP9x associate with cancer and neurodevelopmental disorders. In cancer it impairs normal cell cycle control and apoptosis facilitating unchecked cell proliferation. It interacts with proteins such as MCL1 influencing apoptosis decisions and tumor growth. In neurodevelopmental disorders USP9x affects brain development by disrupting neural cell differentiation processes. The protein's malfunction causes imbalances in brain signaling linking it to conditions like intellectual disability.

Product promise

Tested

We have tested this species and application combination and it works. It is covered by our product promise.

Expected

We have not tested this specific species and application combination in-house, but expect it will work. It is covered by our product promise.

Predicted

This species and application combination has not been tested, but we predict it will work based on strong homology. However, this combination is not covered by our product promise.

Not recommended

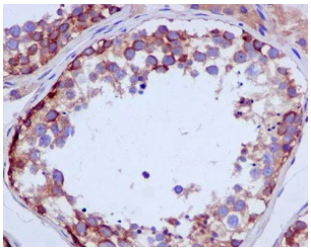
We do not recommend this combination. It is not covered by our product promise.

We are dedicated to supporting your work with high quality reagents and we are here for you every step of the way should you need us.

In the unlikely event of one of our products not working as expected, you are covered by our product promise.

Full details and terms and conditions can be found here:
Terms & Conditions.

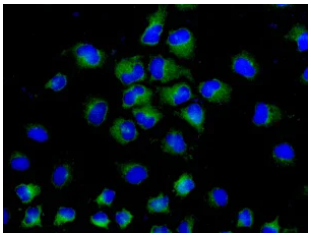
9 product images



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-USP9x antibody [EPR13809(B)] - N-terminal (ab180191)

USP9x Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-USP9x antibody

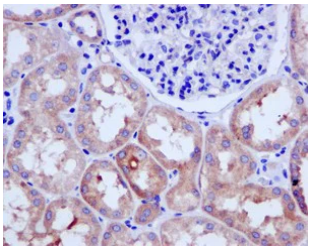
Immunohistochemical analysis of paraffin embedded Human testis tissue labeling USP9x with ab180191 at 1/50.
Perform heat mediated antigen retrieval with EDTA buffer pH 9 before commencing with IHC staining protocol.



Immunocytochemistry/ Immunofluorescence - Anti-USP9x antibody [EPR13809(B)] - N-terminal (ab180191)

USP9x Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-USP9x antibody

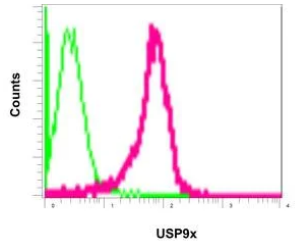
Immunofluorescent analysis of HeLa cells labeling USP9x with ab180191 at 1/100 (green) and DAPI staining (blue).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-USP9x antibody [EPR13809(B)] - N-terminal (ab180191)

USP9x Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-USP9x antibody

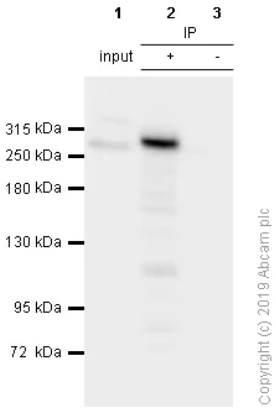
Immunohistochemical analysis of paraffin embedded Human kidney tissue labeling USP9x with ab180191 at 1/50.
Perform heat mediated antigen retrieval with EDTA buffer pH 9 before commencing with IHC staining protocol.



Flow Cytometry (Intracellular) - Anti-USP9x antibody [EPR13809(B)] - N-terminal (ab180191)

USP9x Flow Cytometry (Intracellular) staining using rabbit Anti-USP9x antibody

Intracellular flow cytometrical analysis of permeabilized Jurkat cells labeling USP9x with ab180191 at 1/10 (red) or a rabbit IgG negative (green).



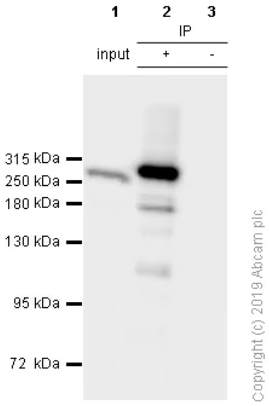
Immunoprecipitation - Anti-USP9x antibody [EPR13809(B)] - N-terminal (ab180191)

ab180191 (purified) at 1/20 dilution immunoprecipitating ETS2 in HeLa (human cervix adenocarcinoma epithelial cell) whole cell lysate 10 µg.
Lane 1 (input): HeLa (human cervix adenocarcinoma epithelial cell) whole cell lysate 10 µg
Lane 2 (+): ab180191 & HeLa whole cell lysate
Lane 3 (-): Rabbit monoclonal IgG (ab172730) instead of ab180191 in HeLa whole cell lysate
For western blotting, ab180191 at 1/500 dilution (0.23 µg/mL) and veriBlot for IP secondary antibody (HRP) (ab131366) at 1/1000 dilution was used.

Blocking and diluting buffer: 5% NFDM /TBST.

All lanes:
Immunoprecipitation - Anti-USP9x antibody [EPR13809(B)] - N-terminal (ab180191)

Predicted band size: 292 kDa



Immunoprecipitation - Anti-USP9x antibody [EPR13809(B)] - N-terminal (ab180191)

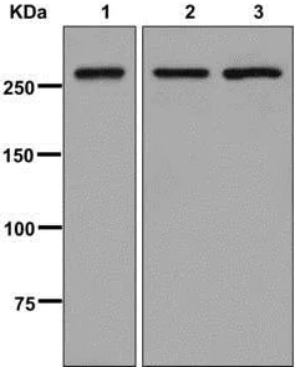
ab180191 (purified) at 1/20 dilution immunoprecipitating ETS2 in NIH/3T3 (Mouse embryonic fibroblast) whole cell lysate 10 µg.

Lane 1 (input): NIH/3T3 (Mouse embryonic fibroblast) whole cell lysate 10 µg.
Lane 2 (+): ab180191 & NIH/3T3 whole cell lysate
Lane 3 (-): Rabbit monoclonal IgG (ab172730) instead of ab180191 in NIH/3T3 whole cell lysate
For western blotting, ab180191 at 1/500 dilution (0.23 µg/mL) and veriBlot for IP secondary antibody (HRP) (ab131366) at 1/1000 dilution was used.

Blocking and diluting buffer: 5% NFDM /TBST.

All lanes:
Immunoprecipitation - Anti-USP9x antibody [EPR13809(B)] - N-terminal (ab180191)

Predicted band size: 292 kDa



Western blot - Anti-USP9x antibody [EPR13809(B)] - N-terminal (ab180191)

All lanes:
Western blot - Anti-USP9x antibody [EPR13809(B)] - N-terminal (ab180191) at 1/1000 dilution

Lane 1:
Jurkat cell lysate at 10 µg

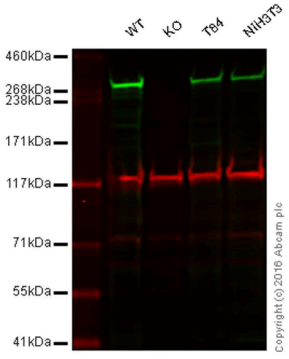
Lane 2:
HeLa cell lysate at 10 µg

Lane 3:
HepG2 cell lysate at 10 µg

Secondary

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

Predicted band size: 292 kDa



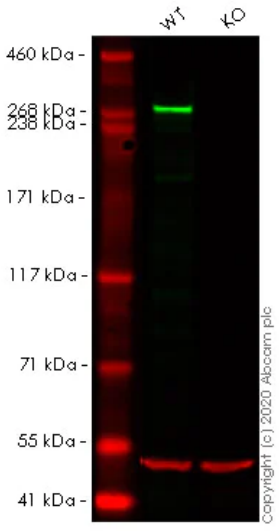
Western blot - Anti-USP9x antibody [EPR13809(B)] - N-terminal (ab180191)

USP9x Western blot staining using rabbit Anti-USP9x antibody

Lane 1: Wild-type HAP1 cell lysate (20 µg)
Lane 2: USP9x knockout HAP1 cell lysate (20 µg)
Lane 3: T84 cell lysate (20 µg)
Lane 4: NIH3T3 cell lysate (20 µg)
Lanes 1 - 4: Merged signal (red and green). Green - ab180191 observed at 270 kDa. Red - loading control, ab18058, observed at 117 kDa.
ab180191 was shown to specifically react with USP9x when USP9x knockout samples were used. Wild-type and USP9x knockout samples were subjected to SDS-PAGE. ab180191 and ab18058 (loading control to Vinculin) were diluted at 1 µg/ml and 1/10 000 respectively and incubated overnight at 4°C. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (ab216773) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preadsorbed (ab216776) secondary antibodies at 1/10 000 dilution for 1 h at room temperature before imaging.

All lanes:
Western blot - Anti-USP9x antibody [EPR13809(B)] - N-terminal (ab180191)

Predicted band size: 292 kDa



Western blot - Anti-USP9x antibody [EPR13809(B)] - N-terminal (ab180191)

USP9x Western blot staining using rabbit Anti-USP9x antibody

Lanes 1- 2: Merged signal (red and green). Green - ab180191 observed at 290 kDa. Red - Anti-alpha Tubulin antibody [DM1A] - Loading Control (ab7291) observed at 50 kDa.
ab180191 was shown to react with USP9x in wild-type HeLa cells in western blot. Loss of signal was observed when knockout cell line ab265665 (knockout cell lysate ab257790) was used. Wild-type HeLa and USP9X knockout HeLa cell lysates were subjected to SDS-PAGE. Membrane was blocked for 1 hour at room temperature in 0.1% TBST with 3% non-fat dried milk. ab180191 and Anti-alpha Tubulin antibody [DM1A] - Loading Control (ab7291) were incubated overnight at 4°C at a 1 in 1000 dilution and a 1 in 20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye®800CW) preadsorbed (ab216773) and Goat anti-Mouse IgG H&L (IRDye®680RD) preadsorbed (ab216776) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.

All lanes:
Western blot - Anti-USP9x antibody [EPR13809(B)] - N-terminal (ab180191) at 1/1000 dilution

Lane 1:
Wild-type HeLa cell lysate at 20 µg

Lane 2:
Western blot - Human USP9X knockout HeLa cell lysate (ab257790) at 20 µg

Performed under reducing conditions.

Predicted band size: 140 kDa, 292 kDa

Observed band size: 220 kDa, 290 kDa

Please note: All products are 'FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC OR THERAPEUTIC PROCEDURES'.