abcam

Product datasheet

Anti-USP10 antibody [EPR4261] ab109219





★★★★★ 1 Abreviews 7 References 6 Images

Overview

Product name Anti-USP10 antibody [EPR4261]

Description Rabbit monoclonal [EPR4261] to USP10

Host species Rabbit

Tested applications Suitable for: WB, IP, IHC-P, ICC/IF

Unsuitable for: Flow Cyt

Species reactivity Reacts with: Human

Immunogen Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.

Positive control WB: HeLa, 293T, A375 and A549 cell lysates. IHC-P: Human colon tissue. ICC: MCF-7 IP: HeLa.

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**.

Mouse, Rat: We have preliminary internal testing data to indicate this antibody may not react with

these species. Please contact us for more information.

Properties

Form Liquid

Storage instructions Shipped at 4°C. Store at -20°C. Stable for 12 months at -20°C.

Storage buffer

Preservative: 0.05% Sodium azide

Constituents: 0.1% BSA, 40% Glycerol (glycerin, glycerine), 9.85% Tris glycine, 50% Tissue

culture supernatant

Purity Tissue culture supernatant

Clonality Monoclonal

Clone number EPR4261

Isotype IgG

Applications

The Abpromise guarantee

Our <u>Abpromise guarantee</u> covers the use of ab109219 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 - 1/10000. Detects a band of approximately 110 kDa (predicted molecular weight: 87 kDa).
IP		1/10 - 1/100.
IHC-P		1/250 - 1/500. Perform heat mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol. Antigen retrieval is recommended.
ICC/IF		1/250 - 1/500.

Application notes

Is unsuitable for Flow Cyt.

Target

Function Hydrolase that can remove conjugated ubiquitin from target proteins such as p53/TP53, SNX3

and CFTR. Acts as an essential regulator of p53/TP53 stability: in unstressed cells, specifically deubiquitinates p53/TP53 in the cytoplasm, leading to counteract MDM2 action and stabilize p53/TP53. Following DNA damage, translocates to the nucleus and deubiquitinates p53/TP53, leading to regulate the p53/TP53-dependent DNA damage response. Does not deubiquitinate

MDM2. Deubiquitinates CFTR in early endosomes, enhancing its endocytic recycling.

Tissue specificity Widely expressed.

Sequence similaritiesBelongs to the peptidase C19 family. USP10 subfamily.

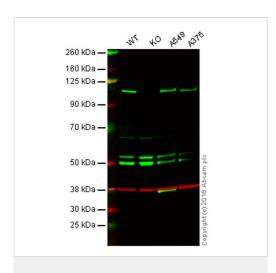
Post-translational Phosphorylated by ATM following DNA damage, leading to stablization and translocation it to the

modifications nucleu

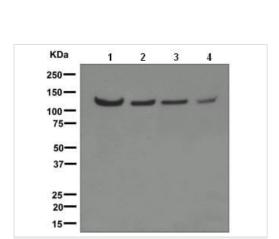
Cellular localization Cytoplasm. Nucleus. Early endosome. Cytoplasmic in normal conditions. After DNA damage,

translocates to the nucleus following phosphorylation by ATM.

Images



Western blot - Anti-USP10 antibody [EPR4261] (ab109219)



Western blot - Anti-USP10 antibody [EPR4261] (ab109219)

Lane 1: Wild-type HAP1 cell lysate (20 µg)

Lane 2: USP10 knockout HAP1 cell lysate (20 µg)

Lane 3: A549 cell lysate (20 µg)

Lane 4: A375 cell lysate (20 µg)

Lanes 1 to 4: Merged signal (red and green). Green - ab109219 observed at 115 kDa. Red - loading control, <u>ab8245</u>, observed at 37 kDa.

ab109219 was shown to recognize USP10 when USP10 knockout samples were used, along with additional cross-reactive bands. Wild-type and USP10 knockout samples were subjected to SDS-PAGE. ab109219 and <u>ab8245</u> (loading control to GAPDH) were both diluted at 1/1000 and 1/10000 respectively and incubated overnight at 4°C. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (<u>ab216773</u>) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preadsorbed (<u>ab216776</u>) secondary antibodies at 1/10000 dilution for 1 h at room temperature before imaging.

All lanes : Anti-USP10 antibody [EPR4261] (ab109219) at 1/1000 dilution

Lane 1: Hela cell lysate

Lane 2: 293T cell lysate

Lane 3: A375 cell lysate

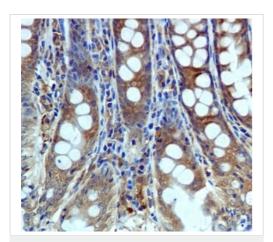
Lane 4: A549 cell lysate

Lysates/proteins at 10 µg per lane.

Secondary

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

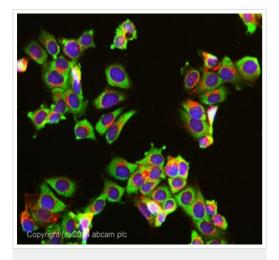
Predicted band size: 87 kDa **Observed band size:** 110 kDa



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-USP10 antibody
[EPR4261] (ab109219)

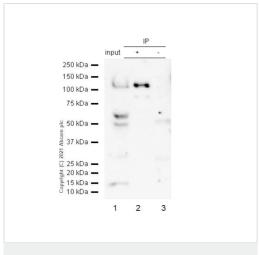
ab109219, at a 1/250 dilution, staining USP10 in paraffin embedded Human colon tissue by Immunohistochemistry.

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



Immunocytochemistry/ Immunofluorescence - Anti-USP10 antibody [EPR4261] (ab109219)

ICC/IF image of ab109219 stained MCF-7 cells. The cells were 100% methanol fixed (5 min) and then incubated in 1%BSA / 10% normal goat serum / 0.3M glycine in 0.1% PBS-Tween for 1h to permeabilise the cells and block non-specific protein-protein interactions. The cells were then incubated with the antibody ab109219 at 1/50 dilution overnight at +4°C. The secondary antibody (pseudo-colored green) was Alexa Fluor® 488 goat antirabbit (ab150081) lgG (H+L) preadsorbed, used at a 1/1000 dilution for 1h. Alexa Fluor® 594 WGA was used to label plasma membranes (pseudo-colored red) at a 1/200 dilution for 1h at room temperature. DAPI was used to stain the cell nuclei (pseudo-colored blue) at a concentration of 1.43 μ M for 1hour at room temperature.



Immunoprecipitation - Anti-USP10 antibody [EPR4261] (ab109219)

USP10 was immunoprecipitated from 0.35 mg HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysate 10 μ g with 109219 at 1/30 dilution (2 μ g) . VeriBlot for IP Detection Reagent (HRP) (ab131366) was used at 1/5000 dilution.

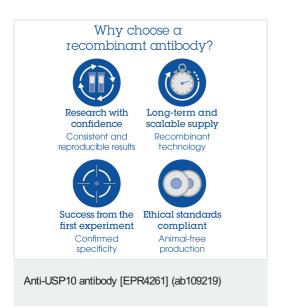
Lane 1: HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysate 10 μg

Lane 2: ab109219 IP in HeLa whole cell lysate

Lane 3: Rabbit monoclonal $\lg G$ ($\underline{ab172730}$) instead of ab109219 in HeLa whole cell lysate

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

Fresh lysate should be used to minimize protein degradation.



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