abcam

ab225537

Anti-USP28 antibody [EPR4249(2)] - BSA and Azide free

Recombinant | RabMAb | KO Validated

Key facts

Isotype	IgG
Host species	Rabbit
Storage buffer	pH: 7.2 - 7.4 Constituents: PBS
Form	Liquid
Clonality	Monoclonal
Immunogen	The exact immunogen used to generate this antibody is proprietary information.
Carrier free	Yes
Clone number	EPR4249(2)
Purification technique	Affinity purification Protein A
Dissociation constant	1.41 x 10^{-11} M
Concentration	1.003 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

IP

Not recommended

Species	Human

Dilution info	-
Notes	-

WB

Tested

Species	Human
Dilution info	-
Notes	-

IHC-P

Not recommended

Species	Human
Dilution info	-
Notes	-

ICC/IF

Tested

Species	Human
Dilution info	-
Notes	-

Flow Cyt (Intra)

Tested

Species	Human
Dilution info	-
Notes	ab199376 - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.

Target data

See full target information USP28

Function	Deubiquitinase involved in DNA damage response checkpoint and MYC proto-oncogene stability. Involved in DNA damage induced apoptosis by specifically deubiquitinating proteins of the DNA damage pathway such as CLSPN. Also involved in G2 DNA damage checkpoint, by deubiquitinating CLSPN, and preventing its degradation by the anaphase promoting complex/cyclosome (APC/C). In contrast, it does not deubiquitinate PLK1. Specifically deubiquitinates MYC in the nucleoplasm, leading to prevent MYC degradation by the proteasome: acts by specifically interacting with isoform 1 of FBXW7 (FBW7alpha) in the nucleoplasm and counteracting ubiquitination of MYC by the SCF(FBW7) complex. In contrast, it does not interact with isoform 4 of FBXW7 (FBW7gamma) in the nucleolus, allowing MYC degradation and explaining the selective MYC degradation in the nucleolus. Deubiquitinates ZNF304, hence preventing ZNF304 degradation by the proteasome and leading to the activated KRAS-mediated promoter hypermethylation and transcriptional silencing of tumor suppressor genes (TSGs) in a subset of colorectal cancers (CRC) cells (PubMed:24623306).

Storage

Shipped at conditions	Blue Ice
Appropriate short-term storage conditions	+4°C
Appropriate long-term storage conditions	+4°C
Storage information	Do Not Freeze

Notes

ab225537 is the carrier-free version of ab126604.

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.

Conjugation ready

Our carrier-free antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. This conjugation-ready format is designed for use with fluorochromes, metal isotopes, oligonucleotides, and enzymes, which makes them ideal for antibody labelling, functional and cell-based assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications.

Use our conjugation kits for antibody conjugates that are ready-to-use in as little as 20 minutes with 1 minute hands-on-time and 100% antibody recovery: available for fluorescent dyes, HRP, biotin and gold.

Compatibility

This product is compatible with the Maxpar® Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar® is a trademark of Fluidigm Canada Inc.

Supplementary info

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

Activity summary	USP28 also known as Ubiquitin Specific Peptidase 28 is a deubiquitinating enzyme responsible for removing ubiquitin from protein substrates. It belongs to the cysteine protease family and has a molecular mass of approximately 120 kDa. USP28 is mainly expressed in the nucleus and cytoplasm of cells and appears in various tissues including the lung liver and gastrointestinal tract. It plays a role in regulating protein stability by counteracting ubiquitination an important process for protein degradation.
Biological function summary	This deubiquitinase regulates several cellular processes such as DNA damage response cell cycle progression and apoptosis. USP28 influences protein stability by directly associating with components of the c-MYC signaling pathway. It is known to interact with and stabilize Fbw7 which is part of the ubiquitin ligase complex thereby affecting the turnover of proteins like c-MYC and cyclin E. Through these interactions USP28 helps maintain normal levels of these important regulators for healthy cellular functions.
Pathways	The enzyme plays an important part in the DNA damage response and MAPK signaling pathways. These pathways ensure proper genomic integrity and cell proliferation. USP28 enhances the stability of the key regulatory protein c-MYC in the p53 pathway emphasizing its role in cell cycle regulation. Its interaction with other proteins like Fbw7 and c-MYC allows it to modulate the rate of cell growth and division indicating its importance in controlled cellular pathways.
Associated diseases and disorders	USP28 has connections to cancer and neurodegenerative diseases. Its interactions with c-MYC are implicated in tumorigenesis particularly in colorectal and breast cancers due to the stabilization of oncogenic proteins that promote uncontrollable cell proliferation. Additionally USP28 might contribute to neurodegenerative disorders by affecting protein aggregates commonly seen in such diseases although studies are less established in this area. The enzyme's strong connection with proteins like Fbw7 and c-MYC highlights its relevance in these disease situations making it a potential target for therapeutic intervention.

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

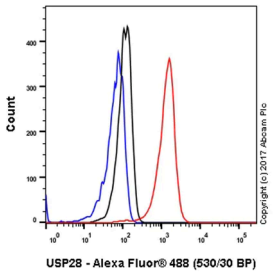
We do not recommend this combination. It is not covered by our product promise.

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.

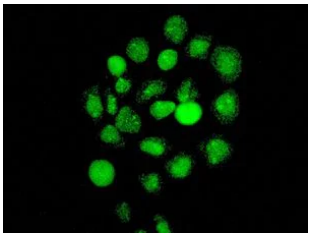
4 product images



Flow Cytometry (Intracellular) - Anti-USP28 antibody [EPR4249(2)] - BSA and Azide free (ab225537)

USP28 Flow Cytometry (Intracellular) staining using rabbit Anti-USP28 antibody

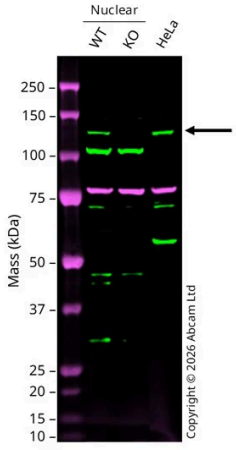
Intracellular Flow Cytometry analysis of HeLa (human cervix adenocarcinoma) cells labeling USP28 with unpurified ab126604 at 1/200 dilution (1ug/ml) (red). Cells were fixed with 4% paraformaldehyde and permeabilised with 90% methanol. A Goat anti rabbit IgG (Alexa Fluor® 488) (ab150077) (1/2000 dilution) was used as the secondary antibody. Rabbit monoclonal IgG (Black) (ab172730) was used as the isotype control, Cell without incubation with primary antibody and secondary antibody (Blue) were used as the unlabeled control.
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab126604).



Immunocytochemistry/ Immunofluorescence - Anti-USP28 antibody [EPR4249(2)] - BSA and Azide free (ab225537)

USP28 Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-USP28 antibody

ab126604 at 1/50 dilution staining USP28 in HeLa cells by Immunofluorescence.This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab126604).



Western blot - Anti-USP28 antibody [EPR4249(2)] - BSA and Azide free (ab225537)

USP28 Western blot staining using rabbit Anti-USP28 antibody

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab126604).

Western blot: Anti-USP28 antibody [EPR4249(2)] ab126604 staining at 1/1000 dilution, shown in green; Mouse anti-CANX ab238078 loading control staining at 1/20000 dilution, shown in magenta. A band was observed at 122 kDa in Wild-type A549 Nuclear cell lysates with no signal observed at this size in USP28 knockout A549 Nuclear cell line. To generate this image, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 3% Milk in TBS-0.1 % Tween® 20 (TBS-T) before incubation with primary antibodies overnight at 4 °C. Blots were washed four times in TBS-T, incubated with secondary antibodies for 1 h at room

temperature, washed again four times then imaged. Secondary antibodies used were Goat anti-Rabbit 800CW and Goat anti-Mouse 680RD at 1/20,000 dilution.
All lanes:
Western blot - Anti-USP28 antibody [EPR4249(2)] (ab126604) at 1/1000 dilution

Lane 1:
Wild-type A549 Nuclear cell lysates at 20 µg

Lane 2:
USP28 knockout A549 Nuclear cell lysates at 20 µg

Lane 3:
HeLa cell lysates at 20 µg

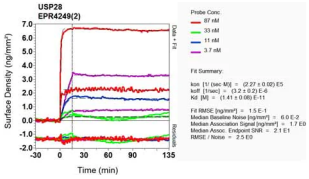
Secondary

All lanes:
Goat anti-Rabbit 800CW & Goat anti-Mouse 680RD at 1/20000 dilution

Performed under reducing conditions.

Predicted band size: 122 kDa

Observed band size: 122 kDa



OI-RD Scanning - Anti-USP28 antibody [EPR4249(2)] - BSA and Azide free (ab225537)

We have systematically measured KD (the equilibrium dissociation constant between the antibody and its antigen), of more than 840 recombinant antibodies to assess not only their individual KD values but also to see the average affinity of antibody. Based on the comparison with published literature values for mouse monoclonal antibodies, Recombinant antibodies appear to be on average 1-2 order of magnitude higher affinity.

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