abcam

Product datasheet

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

KO VALIDATED Recombinant RabMAb

1 References 5 Images

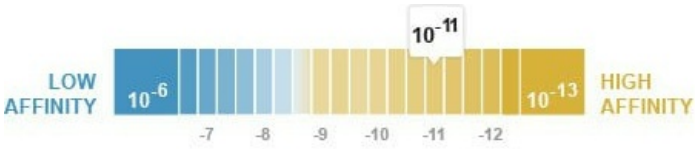
Overview

Product name	Anti-USP28 antibody [EPR4249(2)] - BSA and Azide free
Description	Rabbit monoclonal [EPR4249(2)] to USP28 - BSA and Azide free
Host species	Rabbit
Tested applications	**Suitable for:** Flow Cyt (Intra), ICC/IF, WB **Unsuitable for:** IHC-P or IP
Species reactivity	**Reacts with:** Human
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	293T, HeLa, HT1376, SW480 and A431 cell lysates; Human heart tissue lysate; HeLa cells.
General notes	ab225537 is the carrier-free version of **ab126604**. Our **carrier-free** antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. The carrier-free buffer and high concentration allow for increased conjugation efficiency. 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. 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. 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. Do Not Freeze.

Dissociation constant (K_D) $K_D = 1.41 \times 10^{-11}$ M



Learn more about K_D

Storage buffer pH: 7.20
Constituent: PBS

Carrier free Yes

Purity Protein A purified

Clonality Monoclonal

Clone number EPR4249(2)

Isotype IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab225537 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
Flow Cyt (Intra)		Use at an assay dependent concentration. **ab199376** - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.
ICC/IF		Use at an assay dependent concentration.
WB		Use at an assay dependent concentration. Detects a band of approximately 140 kDa (predicted molecular weight: 122 kDa).

Application notes Is unsuitable for IHC-P or IP.

Target

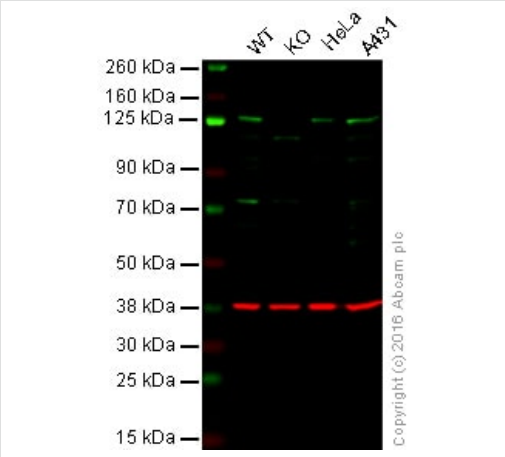
Function Deubiquitinase involved in DNA damage response checkpoint and MYC proto-oncogene stability. Involved in DNA damage induced apoptosis by specifically deubiquitinating proteins of the DNA damage pathway such as CLSPN. Also involved in G2 DNA damage checkpoint, by deubiquitinating CLSPN, and preventing 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.

Sequence similarities Belongs to the peptidase C19 family. USP28 subfamily.
Contains 1 UIM (ubiquitin-interacting motif) repeat.

Post-translational Degradaded upon nickel ion level or hypoxia exposure.

modifications	Phosphorylated upon DNA damage at Ser-67 and Ser-714, by ATM or ATR.
Cellular localization	Nucleus > nucleoplasm.

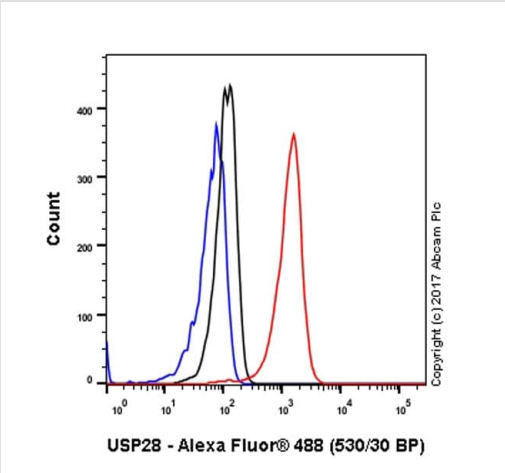
Images



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

This WB data was generated using the same anti-USP28 antibody clone [EPR4249(2)] in a different buffer formulation (cat# **ab126604**).

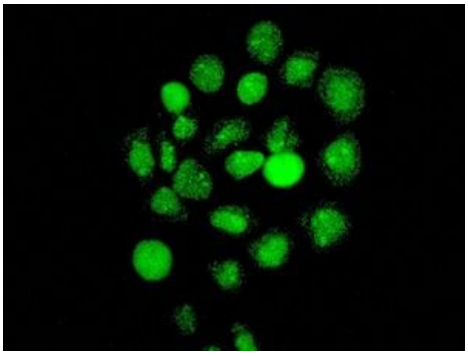
Lane 1: Wild-type HAP1 cell lysate (20 µg)
Lane 2: USP28 knockout HAP1 cell lysate (20 µg)
Lane 3: HeLa cell lysate (20 µg)
Lane 4: A431 cell lysate (20 µg)
Lanes 1 - 4: Merged signal (red and green). Green - **ab126604** observed at 128 kDa. Red - loading control, **ab8245**, observed at 37 kDa.
ab126604 was shown to recognize USP28 when USP28 knockout samples were used, along with additional cross-reactive bands. Wild-type and USP28 knockout samples were subjected to SDS-PAGE. **ab126604** and **ab8245** (loading control to GAPDH) were diluted 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 (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preadsorbed (**ab216776**) secondary antibodies at 1/10000 dilution for 1 hour at room temperature before imaging.



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

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.

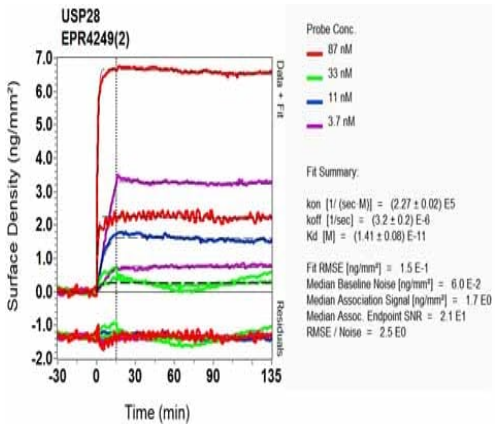
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab126604**).



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

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**).



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

Equilibrium disassociation constant (K_D)

Learn more about K_D

Click here to learn more about K_D

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