abcam

ab309155

Anti-Ubiquitin (phospho S65) antibody [30H3/30K1]

Recombinant | RabMAb

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	30H3/30K1
Purification technique	Affinity purification Protein A
Concentration	0.967 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

WB

Tested

Species	Human
Dilution info	1/1000
Notes	-

Species	Mouse

Dilution info	1/1000
Notes	-

Not recommended

Species	Synthetic peptide - Human, Synthetic peptide
Dilution info	-
Notes	-

ICC/IF

Tested

Species	Human
Dilution info	1/250
Notes	-

Expected

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

Not recommended

Species	Synthetic peptide - Human, Synthetic peptide
Dilution info	-
Notes	-

Flow Cyt (Intra)

Tested

Species	Human
Dilution info	1/1000
Notes	-

Expected

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

Not recommended

Species	Synthetic peptide - Human, Synthetic peptide
Dilution info	-
Notes	-

I-ELISA

Tested

Species	Synthetic peptide - Human
Dilution info	1/1000
Notes	-

Expected

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

Not recommended

Species	Synthetic peptide
Dilution info	-
Notes	-

Dot

Not recommended

Species	Synthetic peptide, Human, Mouse, Synthetic peptide - Human
Dilution info	-
Notes	-

Target data

See full target information UBC pS65

Function	Ubiquitin. Exists either covalently attached to another protein, or free (unanchored). When covalently bound, it is conjugated to target proteins via an isopeptide bond either as a monomer (monoubiquitin), a polymer linked via different Lys residues of the ubiquitin (polyubiquitin chains) or a linear polymer linked via the initiator Met of the ubiquitin (linear polyubiquitin chains). Polyubiquitin chains, when attached to a target protein, have different functions depending on the Lys residue of the ubiquitin that is linked: Lys-6-linked may be involved in DNA repair; Lys-11-linked is involved in ERAD (endoplasmic reticulum-associated degradation) and in cell-cycle regulation; Lys-29-linked is involved in proteotoxic stress response and cell cycle; Lys-33-linked is involved in kinase modification; Lys-48-linked is involved in protein degradation via the proteasome; Lys-63-linked is involved in endocytosis, DNA-damage responses as well as in signaling processes leading to activation of the transcription factor NF-kappa-B. Linear polymer chains formed via attachment by the initiator Met lead to cell signaling. Ubiquitin is usually conjugated to Lys residues of target proteins, however, in rare cases, conjugation to Cys or Ser residues has been observed. When polyubiquitin is free (unanchored-polyubiquitin), it also has distinct roles, such as in activation of protein kinases, and in signaling. During ubiquitination, the acceptor ubiquitin is positioned in the active site via direct interaction with the E2 ubiquitin-conjugating enzymes such as UBE2R2 (PubMed:38326650). As a monoubiquitin, its C-terminal glycine is recognized as a C-degron by Cul2-RING (CRL2) E3 ubiquitin-protein ligase complexes (PubMed:39548056).

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

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.

Collaborations
This antibody was developed with support from The Michael J. Fox Foundation.



Want a custom formulation?
This antibody clone is manufactured by Abcam. If you require a custom buffer formulation or conjugation for your experiments, please contact orders@abcam.com

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.

Supplementary info

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

Activity summary	Ubiquitin is a small regulatory protein found in almost all tissues of eukaryotic organisms. It has a molecular weight of approximately 8.6 kilodaltons. It functions mechanically by attaching to proteins through a process called ubiquitination which involves the formation of an isopeptide bond. Ubiquitin molecules can form polyubiquitin chains through different lysine residues such as K48 and K63 that determine their function. These chains label substrate proteins for various fates including degradation. Ubiquitin is expressed ubiquitously in cells reflecting its essential role in maintaining protein homeostasis.
Biological function summary	The ubiquitin system plays a critical role in regulating protein turnover and quality control within cells. It is part of a larger complex known as the ubiquitin-proteasome system (UPS) which is responsible for degrading proteins that need to be turned over. This process is essential for cell cycle control response to oxidative stress and DNA repair. Ubiquitin's role in tagging proteins for degradation or signaling allows cells to respond quickly to changes in their environment and maintain balance.
Pathways	Ubiquitin functions in several important biological pathways including the Wnt and NF-kB pathways. In the Wnt signaling pathway ubiquitination modulates the stability of key components thereby affecting the pathway's overall activity. In the NF-kB signaling pathway ubiquitin labels inhibitor proteins for degradation which releases and activates NF-kB. These pathways highlight ubiquitin's interaction with

	proteins such as beta-catenin in Wnt and IkB in NF-kB illustrating how it regulates diverse cellular processes.
Associated diseases and disorders	The dysfunction of the ubiquitin system is linked to neurodegenerative diseases and cancers. Ubiquitin-related defects in protein degradation can lead to the buildup of unwanted proteins contributing to conditions like Parkinson's disease. Connections with cancer are evident as ubiquitin controls cell cycle proteins and aberrant ubiquitination may drive tumor growth and progression. The protein p53 known to be controlled by ubiquitination plays a significant role in cancer related mechanisms when dysregulated. Understanding and targeting ubiquitin-related pathways may provide new therapeutic opportunities for treating these conditions.

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

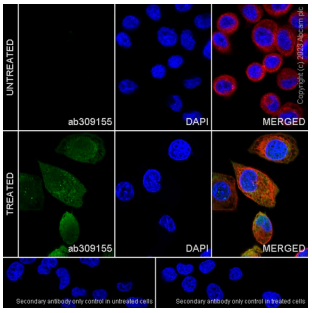
We do not recommend this combination. It is not covered by our product promise.

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.

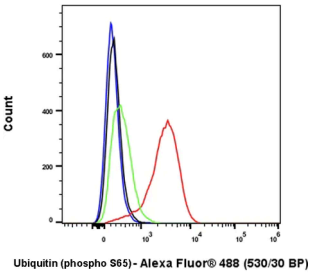
Full details and terms and conditions can be found here:
Terms & Conditions.

6 product images



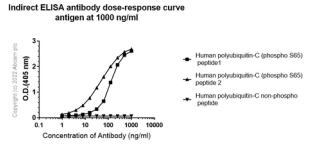
Immunocytochemistry/ Immunofluorescence - Anti-Ubiquitin (phospho S65) antibody [30H3/30K1] (ab309155)

Immunofluorescent analysis of 4% Paraformaldehyde-fixed, 0.1% TritonX-100 permeabilized PC-3 (human prostate adenocarcinoma epithelial cell) cells labelling Ubiquitin (phospho S65) with ab309155 at 1/250 dilution (3.652 ug/ml), followed by ab150081 Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed antibody at 1/1000 dilution (2 ug/ml) (Green). Confocal image showing cytoplasmic and nuclear staining in PC-3 cells treated with CCCP (30µM) (ab141229) for 6 h. Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8). ab195889 Anti-alpha Tubulin mouse monoclonal antibody - Microtubule Marker (Alexa Fluor® 594) was used to counterstain tubulin at 1/200 dilution (2.5 ug/ml) (Red). The nuclear counterstain was DAPI (Blue). Secondary antibody only control: Secondary antibody is ab150081 Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed at 1/1000 dilution (2 ug/ml).



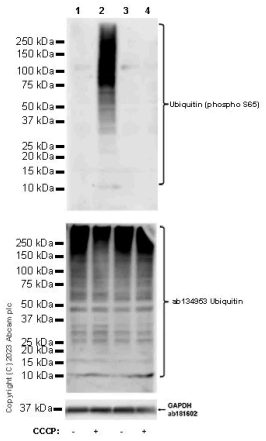
Flow Cytometry (Intracellular) - Anti-Ubiquitin (phospho S65) antibody [30H3/30K1] (ab309155)

Flow cytometric analysis of 4% paraformaldehyde fixed 90% methanol permeabilized PC-3 cells (human pancreas adenocarcinoma epithelial cells) treated with 30µM CCCP (ab141229) for 6 hours (Red) / untreated PC-3 cells (Green) cells labelling Ubiquitin (phospho S65) with ab309155 at 1/1000 dilution (0.1 ug) compared with a Rabbit monoclonal IgG (ab172730) (Black) isotype control and an unlabelled control (cells without incubation with primary antibody and secondary antibody) (Blue). A Goat Anti-Rabbit IgG (Alexa Fluor® 488, ab150081) at 1/5000 dilution was used as the secondary antibody.



Indirect ELISA - Anti-Ubiquitin (phospho S65) antibody [30H3/30K1] (ab309155)

Indirect ELISA analysis of ab309155 at 0-1000 ng/ml. The secondary antibody used was Alkaline Phosphatase-conjugated AffiniPure Goat Anti-Rabbit IgG (H+L) at 1/2500 dilution. Antigen: Human polyubiquitin-C (phospho S65) peptide1, Human polyubiquitin-C (phospho S65) peptide 2,Human polyubiquitin-C non-phospho peptide. Antigen concentration: 1000 ng/ml



Western blot - Anti-Ubiquitin (phospho S65) antibody [30H3/30K1] (ab309155)

Blocking and diluting buffer and concentration: 5% NFDM/TBST. In Western blot, Anti-Ubiquitin antibody (ab134953) staining at 1/1000 dilution. Anti-GAPDH antibody (ab181602) loading control staining at 1/200000 dilution. Exposure time: 97 seconds.
All lanes:
Western blot - Anti-Ubiquitin (phospho S65) antibody [30H3/30K1] (ab309155) at 1/1000 dilution

Lane 1:
Untreated Wild-type HEK-293T (human embryonic kidney epithelial cell) whole cell lysate at 20 µg

Lane 2:
HEK-293T treated with 20 uM CCCP (ab141229) for 8 hours, whole cell lysate at 20 µg

Lane 3:
Untreated PINK1 knockout HEK-293T whole cell lysate at 20 µg

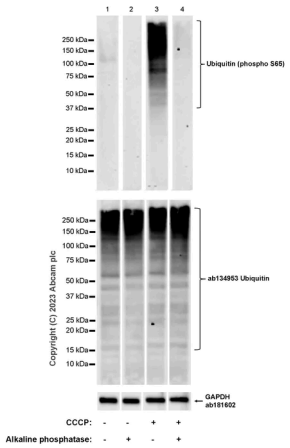
Lane 4:
PINK1 knockout HEK-293T treated with 20 uM CCCP (ab141229) for 8 hours, whole cell lysate at 20 µg

Secondary

All lanes:
Western blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/100000 dilution

Observed band size: 8 kDa, 200 kDa

Exposure time: 97s



Western blot - Anti-Ubiquitin (phospho S65) antibody [30H3/30K1] (ab309155)

Blocking and diluting buffer and concentration: 5% NFDM/TBST. The lysates were freshly made and used for Western Blotting immediately to minimize protein degradation. In Western blot, Anti-Ubiquitin antibody (ab134953) staining at 1/1000 dilution. Anti-GAPDH antibody (ab181602) loading control staining at 1/200000 dilution. Exposure time: 180 seconds.
All lanes:
Western blot - Anti-Ubiquitin (phospho S65) antibody [30H3/30K1] (ab309155) at 1/1000 dilution

Lane 1:
Untreated PC-3 (human prostate adenocarcinoma epithelial cell) whole cell lysate (untreated membrane) at 20 µg

Lane 2:
Untreated PC-3 whole cell lysate (Alkaline phosphatase treated membrane) at 20 µg

Lane 3:
PC-3 treated with 30 uM CCCP (ab141229) for 2 hours, whole cell lysate (untreated membrane) at 20 µg

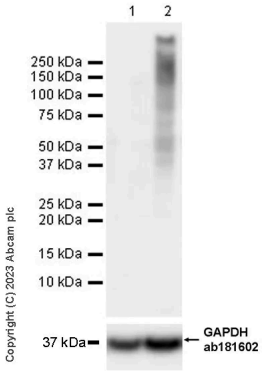
Lane 4:
PC-3 treated with 30 uM CCCP (ab141229) for 2 hours, whole cell lysate (Alkaline phosphatase treated membrane) at 20 µg

Secondary

All lanes:
Western blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/100000 dilution

Observed band size: 8 kDa, 200 kDa

Exposure time: 180s



Western blot - Anti-Ubiquitin (phospho S65) antibody [30H3/30K1] (ab309155)

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

ab181602 was used as GAPDH loading control.

All lanes:
Western blot - Anti-Ubiquitin (phospho S65) antibody [30H3/30K1] (ab309155) at 1/1000 dilution

Lane 1:
Untreated MEF (mouse embryo fibroblast) whole cell lysate at 20 µg

Lane 2:

MEF treated with 5 µM Valinomycin for 24 hours whole cell lysate at 20 µg

Secondary

All lanes:
Western blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/100000 dilution

Predicted band size: 26 kDa

Observed band size: 8-200 kDa, 27 kDa

Exposure time: 26s

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