abcam

ab221211

Anti-Ubiquitin (linkage-specific K48) antibody [EP8589] - Low endotoxin, Azide free

Recombinant RabMAb

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	EP8589
Purification technique	Affinity purification Protein A
Specificity	This antibody only recognizes polyubiquitin chains formed by Lys-48 (K48) residue linkage. This antibody can detect the target in mouse and rat cell lines and induced tissues.
Concentration	1.047 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

WB

Tested

Species	Human, Mouse, Rat
Dilution info	-
Notes	-

ICC/IF

Tested

Species	Human
Dilution info	-
Notes	-

Expected

Species	Mouse, Rat
Dilution info	Use at an assay dependent concentration.
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.

Expected

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

IHC-P

Tested

Species	Human
Dilution info	-
Notes	-

Not recommended

Species	Mouse, Rat
Dilution info	-
Notes	-

Target data

See full target information UBC Linkage-specific K48

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	Blue Ice
Appropriate short-term storage conditions	+4°C

Appropriate long-term storage conditions	+4°C
Storage information	Do Not Freeze

Notes

ab221211 is the carrier-free version of ab140601.
The mouse and rat recommendation is based on the WB results. This antibody may not be suitable for IHC with mouse or rat samples.

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.

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.

What does low endotoxin mean?
Our low endotoxin, azide-free formats have low endotoxin level (1 EU/mg, determined by the TAL assay) and are free from azide, to achieve consistent experimental results in functional assays.

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

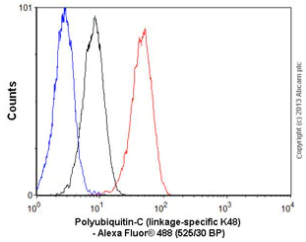
We do not recommend this combination. It is not covered by our product promise.

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:

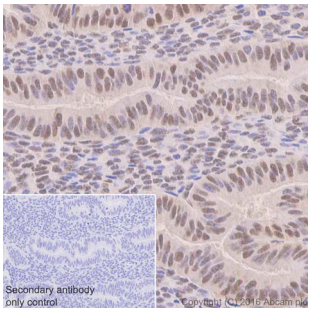
4 product images



Flow Cytometry (Intracellular) - Anti-Ubiquitin (linkage-specific K48) antibody [EP8589] - Low endotoxin, Azide free (ab221211)

Ubiquitin (linkage-specific K48) Flow Cytometry (Intracellular) staining using rabbit Anti-Ubiquitin (linkage-specific K48) antibody

Overlay histogram showing HeLa cells stained with ab140601 (red line). The cells were fixed with 80% methanol (5 min) and then permeabilized with 0.1% PBS-Tween for 20 min. The cells were then incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions followed by the antibody (ab140601, 1/100 dilution) for 30 min at 22°C. The secondary antibody used was Alexa Fluor® 488 goat anti-rabbit IgG (H&L) (ab150077) at 1/2000 dilution for 30 min at 22°C. Isotype control antibody (black line) was rabbit IgG (monoclonal) (1µg/1x10^6 cells) used under the same conditions. Unlabelled sample (blue line) was also used as a control. Acquisition of >5,000 events were collected using a 20mW Argon ion laser (488nm) and 525/30 bandpass filter. This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab140601).

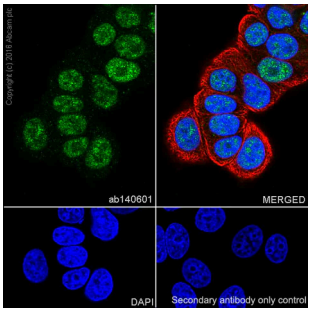


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Ubiquitin (linkage-specific K48) antibody [EP8589] - Low endotoxin, Azide free (ab221211)

Ubiquitin (linkage-specific K48) Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-Ubiquitin (linkage-specific K48) antibody

Purified ab140601 staining Ubiquitin (linkage-specific K48) in human endometrium carcinoma tissue sections by Immunohistochemistry (IHC-P - paraformaldehyde-fixed paraffin-embedded sections). Tissue was fixed with paraformaldehyde and antigen retrieval was by heat mediation in a EDTA buffer. Samples were incubated with primary antibody at a dilution of 1/250. A goat anti-rabbit IgG H&L (HRP) ab97051 was used as the secondary antibody at a dilution of 1/500.
Negative control 1: PBS in place of primary antibody.

This data was developed using the same antibody clone in a different buffer formulation containing PBS BSA glycerol and sodium azide (ab140601).

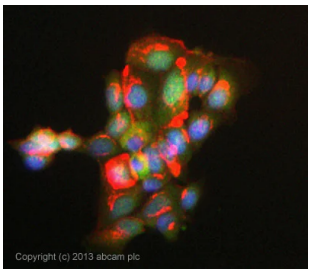


Immunocytochemistry/ Immunofluorescence - Anti-Ubiquitin (linkage-specific K48) antibody [EP8589] - Low endotoxin, Azide free (ab221211)

Ubiquitin (linkage-specific K48) Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-Ubiquitin (linkage-specific K48) antibody

Purified ab140601 staining Ubiquitin (linkage-specific K48) in MCF7 (Human breast adenocarcinoma cell line) cells by ICC/IF (Immunocytochemistry/immunofluorescence). Cells were fixed with 4% Paraformaldehyde and permeabilized with 0.1% Triton X-100. Samples were incubated with primary antibody at a dilution of 1/500. A goat anti rabbit IgG (Alexa Fluor® 488) (ab150077) was used as the secondary antibody at a dilution of 1/1000. ab195889 was used as a

counterstain for primary antibody ab133645 at 1/2000. DAPI was used as a nuclear counterstain and PBS as a negative control.
This data was developed using the same antibody clone in a different buffer formulation containing PBS BSA glycerol and sodium azide (ab140601).



Immunocytochemistry/ Immunofluorescence - Anti-Ubiquitin (linkage-specific K48) antibody [EP8589] - Low endotoxin, Azide free (ab221211)

Ubiquitin (linkage-specific K48) Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-Ubiquitin (linkage-specific K48) antibody

ICC/IF image of ab140601 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 ab140601 at 10µg/ml overnight at +4°C. The secondary antibody (green) was DyLight® 488 goat anti- rabbit (ab96899) IgG (H+L) used at a 1/250 dilution for 1h. Alexa Fluor® 594 WGA was used to label plasma membranes (red) at a 1/200 dilution for 1h. DAPI was used to stain the cell nuclei (blue) at a concentration of 1.43µM.
This data was developed using the same antibody clone in a different buffer formulation containing PBS BSA glycerol and sodium azide (ab140601).

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