

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

Recombinant RabMAb

5 Images

### Overview

<b>Product name</b>	Anti-Ubiquitin (linkage-specific K48) antibody [EP8589] - Low endotoxin, Azide free
<b>Description</b>	Rabbit monoclonal [EP8589] to Ubiquitin (linkage-specific K48) - Low endotoxin, Azide free
<b>Host species</b>	Rabbit
<b>Specificity</b>	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.
<b>Tested applications</b>	<b>Suitable for:</b> Flow Cyt (Intra), ICC/IF, IHC-P, WB
<b>Species reactivity</b>	<b>Reacts with:</b> Mouse, Rat, Human, Recombinant fragment
<b>Immunogen</b>	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
<b>Positive control</b>	K48-linked-Ub2-7 This antibody gave a positive result when used in the following methanol fixed cell lines: MCF-7
<b>General notes</b>	<p>ab221211 is the carrier-free version of <a href="#">ab140601</a>.</p> <p>The mouse and rat recommendation is based on the WB results. This antibody may not be suitable for IHC with mouse or rat samples.</p> <p>Our <b>carrier-free</b> 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.</p> <p>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.</p> <p>Use our <b>conjugation kits</b> for antibody conjugates that are ready-to-use in as little as 20 minutes with &lt;1 minute hands-on-time and 100% antibody recovery: available for fluorescent dyes, HRP, biotin and gold.</p> <p>This product is a recombinant monoclonal antibody, which offers several advantages including:</p> <ul style="list-style-type: none"> <li>- High batch-to-batch consistency and reproducibility</li> <li>- Improved sensitivity and specificity</li> <li>- Long-term security of supply</li> <li>- Animal-free production</li> </ul> <p>For more information <a href="#">see here</a>.</p> <p>Our RabMAb<sup>®</sup> technology is a patented hybridoma-based technology for making rabbit</p>

monoclonal antibodies. For details on our patents, please refer to [\*\*RabMAb® patents\*\*](#).

Our **Low endotoxin, azide-free formats** have low endotoxin level ( $\leq 1$  EU/ml, determined by the LAL assay) and are free from azide, to achieve consistent experimental results in functional assays.

## Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C. Do Not Freeze.
Storage buffer	pH: 7.2 Constituent: PBS
Carrier free	Yes
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EP8589
Isotype	IgG

## Applications

**The Abpromise guarantee** Our **Abpromise guarantee** covers the use of ab221211 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. <b>ab199376</b> - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.
ICC/IF		Use at an assay dependent concentration.
IHC-P		Use at an assay dependent concentration. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
WB		Use at an assay dependent concentration.

## Target

### Relevance

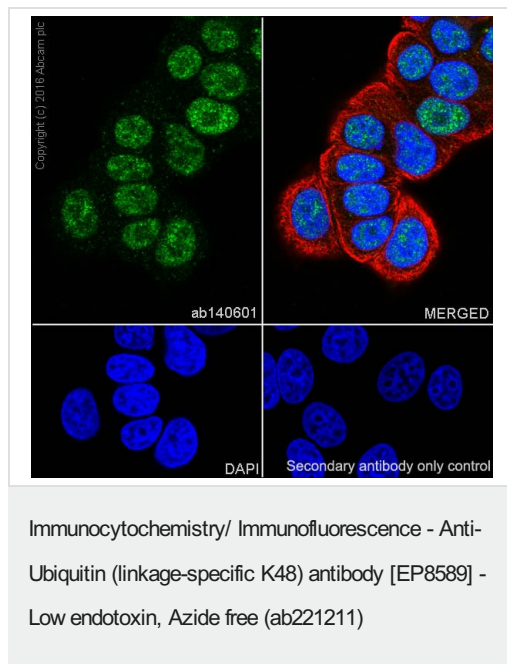
Function: Ubiquitin exists either covalently attached to another protein, or free (unanchored). When covalently bound, it is conjugated to target proteins via an isopeptide bond either as a monomer (monoubiquitin), a polymer linked via different Lys residues of the ubiquitin (polyubiquitin chains) or 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 lysosomal degradation; 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. Similarity: Belongs to the ubiquitin family. Contains 3 ubiquitin-like domains.

## Cellular localization

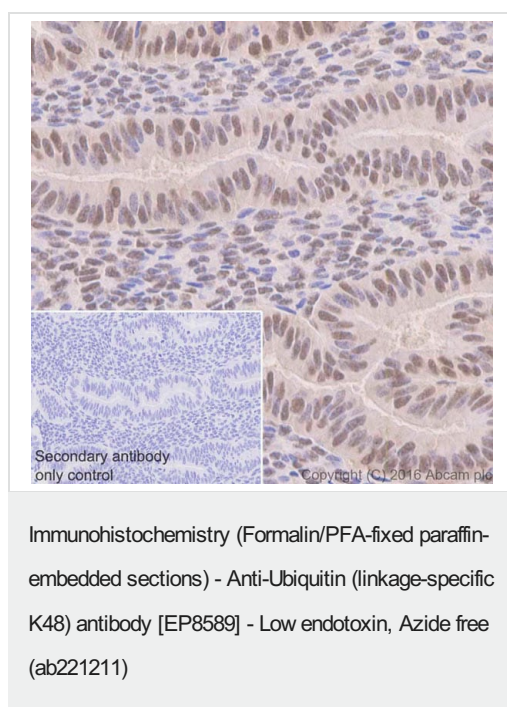
Cell Membrane, Cytoplasmic and Nuclear

## Images



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.

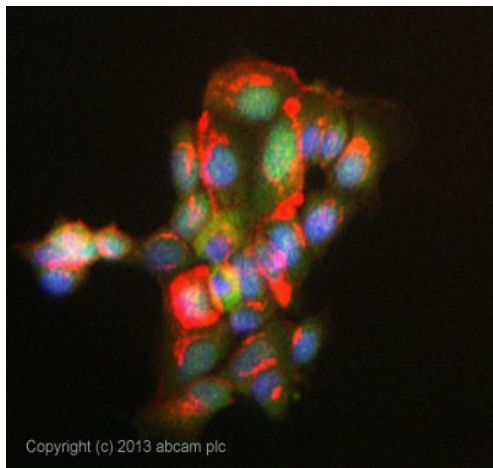
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab140601**).



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.

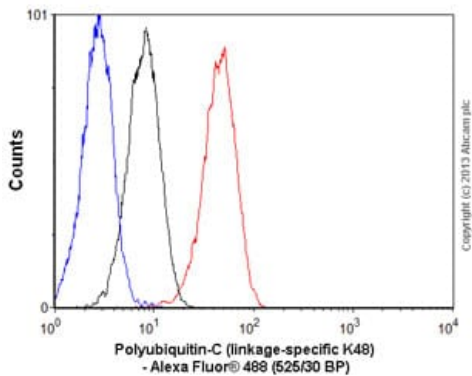
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab140601**).



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

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**).



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

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<sup>6</sup> 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**).

### Why choose a recombinant antibody?



**Research with confidence**  
Consistent and reproducible results



**Long-term and scalable supply**  
Recombinant technology



**Success from the first experiment**  
Confirmed specificity



**Ethical standards compliant**  
Animal-free production

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

**Please note:** All products are "FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES"

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