abcam

Product datasheet

Anti-Ubiquitin (linkage-specific K48) antibody [EP8589] ab140601

Recombinant RabMAb

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Overview

Product name	Anti-Ubiquitin (linkage-specific K48) antibody [EP8589]
Description	Rabbit monoclonal [EP8589] to Ubiquitin (linkage-specific K48)
Host species	Rabbit
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.
Tested applications	**Suitable for:** Flow Cyt (Intra), ICC/IF, IHC-P, WB
Species reactivity	**Reacts with:** Mouse, Rat, Human, Recombinant fragment
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	K48-linked-Ub2-7 This antibody gave a positive result when used in the following methanol fixed cell lines: MCF-7
General notes	The mouse and rat recommendation is based on the WB results. This antibody may not be suitable for IHC with mouse or rat samples. 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 production For more information **see here**. 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. Upon delivery aliquot and store at -20°C. Avoid freeze / thaw cycles.
Storage buffer	Preservative: 0.01% Sodium azide Constituents: 40% Glycerol, 0.05% BSA, 59% PBS
Purity	Protein A purified

Clonality	Monoclonal
Clone number	EP8589
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab140601 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

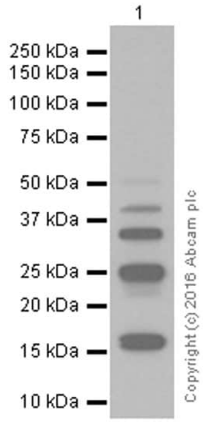
Application	Abreviews	Notes
Flow Cyt (Intra)		1/20 - 1/1000. **ab172730** - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.
ICC/IF	★★★★☆ (2)	1/500.
IHC-P		1/250. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
WB	★★★★★ (7)	1/200 - 1/10000. Detects a band of approximately 17-60 kDa (predicted molecular weight: 77 kDa).

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



Western blot - Anti-Ubiquitin (linkage-specific K48) antibody [EP8589] (ab140601)

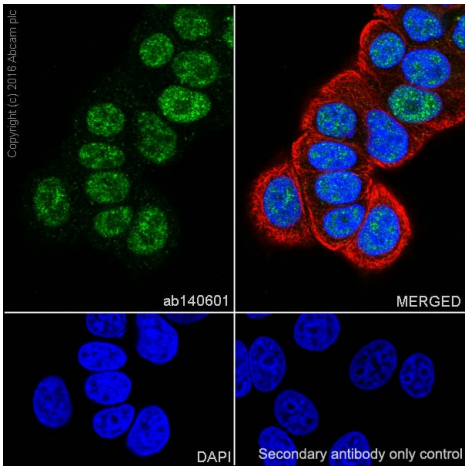
Anti-Ubiquitin (linkage-specific K48) antibody [EP8589] (ab140601) at 1/1000 dilution + K48-linked-Ub2-7recombinant protein lysate at 15 µg

Secondary

Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/20000 dilution

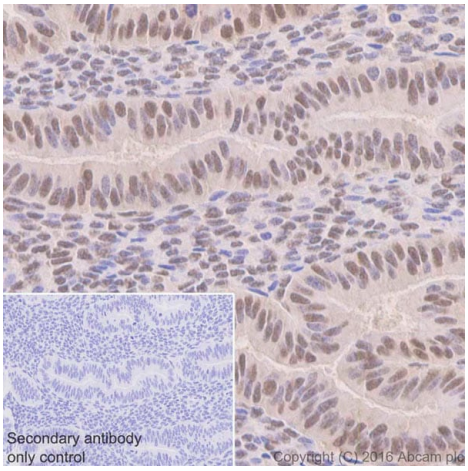
Predicted band size: 77 kDa

Blocking and diluting buffer: 5% NFDM/TBST. This image is produced useing purified ab140601.



Immunocytochemistry/ Immunofluorescence - Anti-Ubiquitin (linkage-specific K48) antibody [EP8589] (ab140601)

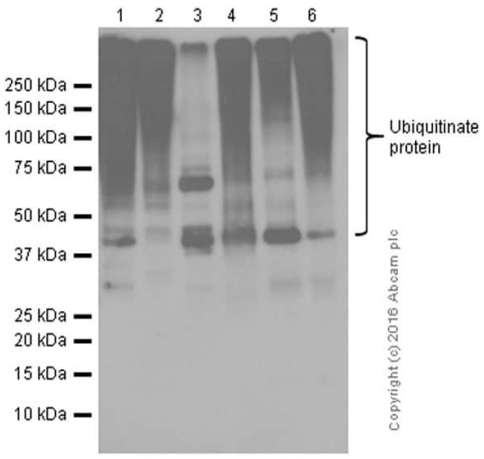
Purified ab140601 staining Ubiquitin (linkage-specific K48) in MCF7 (Human breast adenocarcinoma cell line) cells by ICC/IF (Immunocytochemistry/immunofluorescence). Cells were fixed with 4% Paraformaldehyde and permeabilized with 0.1% Triton X-100. Samples were incubated with primary antibody at a dilution 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.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Ubiquitin (linkage-specific K48) antibody [EP8589] (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.



Western blot - Anti-Ubiquitin (linkage-specific K48) antibody [EP8589] (ab140601)

All lanes : Anti-Ubiquitin (linkage-specific K48) antibody [EP8589] (ab140601) at 200 µg

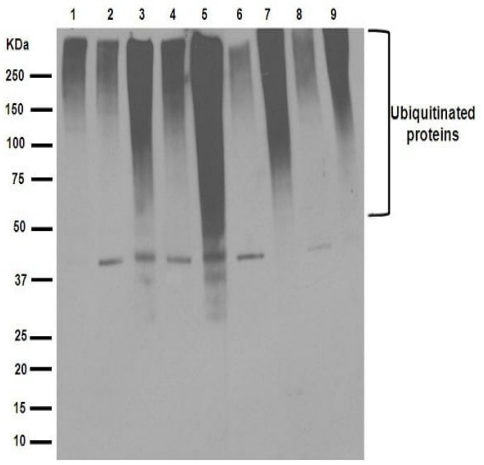
Lane 1 : Jurkat (Human T cell leukemia T lymphocyte) whole cell lysate
Lane 2 : 293 (Human embryonic kidney epithelial cell) whole cell lysate
Lane 3 : Mouse heart lysate
Lane 4 : Rat heart lysate
Lane 5 : C2C12 (Mouse myoblasts myoblast) whole cell lysate
Lane 6 : C6 (Rat glial tumor glial cell) whole cell lysate

Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/20000 dilution

Predicted band size: 77 kDa

Blocking and diluting buffer: 5% NFDM/TBST. This image is produced useing purified ab140601.



Western blot - Anti-Ubiquitin (linkage-specific K48) antibody [EP8589] (ab140601)

All lanes : Anti-Ubiquitin (linkage-specific K48) antibody [EP8589] (ab140601) at 1/200 dilution

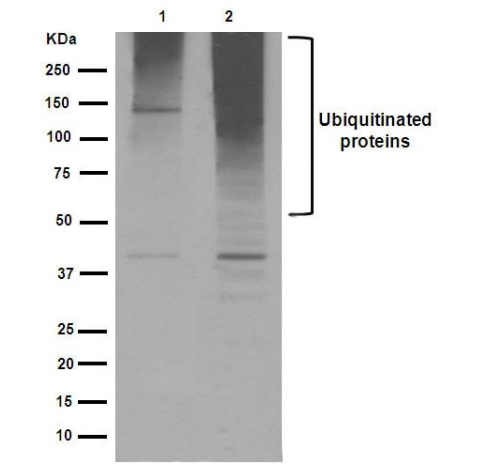
Lane 1 : PC-12
Lane 2 : C6
Lane 3 : L6
Lane 4 : C2C12
Lane 5 : Neuro-2a
Lane 6 : NIH3T3
Lane 7 : SP2/0
Lane 8 : Raw264.7
Lane 9 : B16-F0

Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated at 1/1000 dilution

Predicted band size: 77 kDa

Observed band is above 60kDa



Western blot - Anti-Ubiquitin (linkage-specific K48) antibody [EP8589] (ab140601)

All lanes : Anti-Ubiquitin (linkage-specific K48) antibody [EP8589] (ab140601) at 1/200 dilution

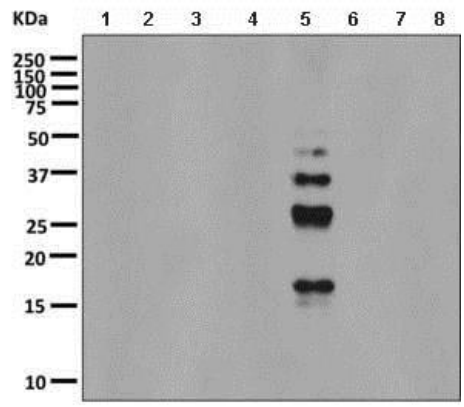
Lane 1 : HEK293
Lane 2 : Jurkat

Lysates/proteins at 10 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated at 1/1000 dilution

Predicted band size: 77 kDa

Observed band is above 60kDa



Western blot - Anti-Ubiquitin (linkage-specific K48) antibody [EP8589] (ab140601)

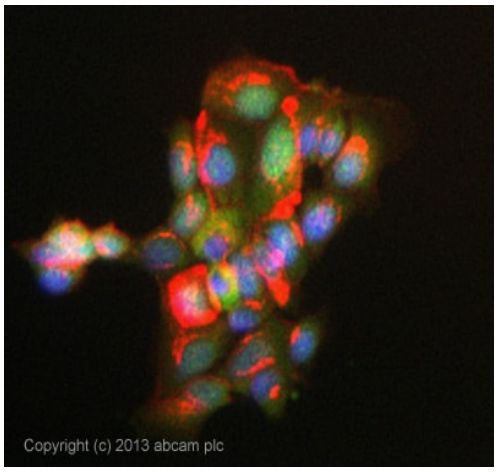
All lanes : Anti-Ubiquitin (linkage-specific K48) antibody [EP8589] (ab140601) at 1/1000 dilution

Lane 1 : K6-linked-Ub2 recombinant protein
Lane 2 : K27-linked-Ub2 recombinant protein
Lane 3 : K29-linked-Ub2 recombinant protein
Lane 4 : K11-linked-Ub2 recombinant protein
Lane 5 : K48-linked-Ub2-7recombinant protein
Lane 6 : K63-linked-Ub2-7 recombinant protein
Lane 7 : K33-linked-Ub2 recombinant protein
Lane 8 : monoubiquitin recombinant protein

Lysates/proteins at 0.01 µg per lane.

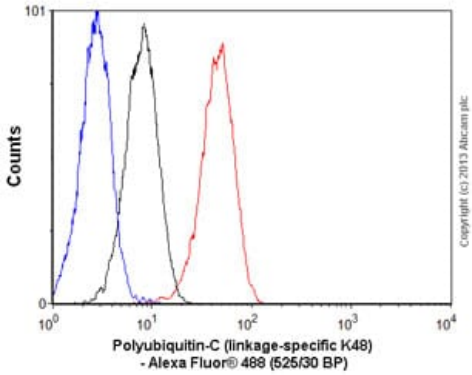
Secondary
All lanes : HRP labelled goat anti-rabbit at 1/2000 dilution

Predicted band size: 77 kDa
Observed band size: 17-60 kDa



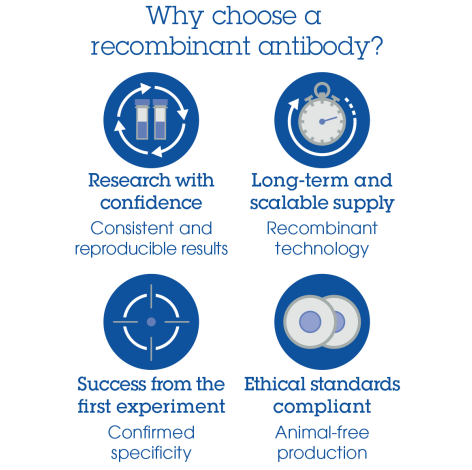
Immunocytochemistry/ Immunofluorescence - Anti-Ubiquitin (linkage-specific K48) antibody [EP8589] (ab140601)

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.



Flow Cytometry (Intracellular) - Anti-Ubiquitin (linkage-specific K48) antibody [EP8589] (ab140601)

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.



Anti-Ubiquitin (linkage-specific K48) antibody [EP8589] (ab140601)

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