

## **Product datasheet**

# Anti-Ubiquitin (linkage-specific K63) antibody [EPR8590-448] ab179434

Recombinant RabMAb

★★★★★ <u>6 Abreviews</u> <u>75 References</u> 8 Images

Overview	
Product name	Anti-Ubiquitin (linkage-specific K63) antibody [EPR8590-448]
Description	Rabbit monoclonal [EPR8590-448] to Ubiquitin (linkage-specific K63)
Host species	Rabbit
Tested applications	Suitable for: Flow Cyt (Intra), WB, IHC-P
Species reactivity	Reacts with: Mouse, Rat, Human
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: K63-linked-Ub2-7 recombinant protein, HEK-293 and HeLa cell lysates, and Mouse and rat brain lysate. IHC-P: Human kidney carcinoma and tonsil tissue. Flow Cyt (intra): HeLa cells.
General notes	<ul> <li>This product is a recombinant monoclonal antibody, which offers several advantages including:</li> <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> <li>For more information <u>see here</u>.</li> <li>Our RabMAb<sup>®</sup> technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to <u>RabMAb<sup>®</sup> patents</u>.</li> </ul>

Properties	
Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C long term. Avoid freeze / thaw cycle.
Storage buffer	Preservative: 0.01% Sodium azide Constituents: 59% PBS, 40% Glycerol, 0.05% BSA
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR8590-448
lsotype	lgG

### Applications

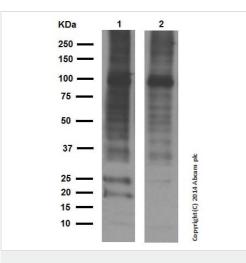
The Abpromise guarantee Our <u>Abpromise guarantee</u> covers the use of ab179434 in the following tested applications.

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

Application	Abreviews	Notes
Flow Cyt (Intra)		1/210.
WB	$\star$ $\star$ $\star$ $\star$ $\star$ (4)	1/1000. Predicted molecular weight: 77 kDa.
IHC-P		1/250 - 1/500. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol. <b>For unpurified, use 1/100 - 1/250.</b>

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



**All lanes :** Anti-Ubiquitin (linkage-specific K63) antibody [EPR8590-448] (ab179434) at 1/1000 dilution (purified)

Lane 1 : HEK-293 (human epithelial cell line from embryonic kidney) cell lysate
Lane 2 : HeLa (human epithelial cell line from cervix adenocarcinoma) cell lysate

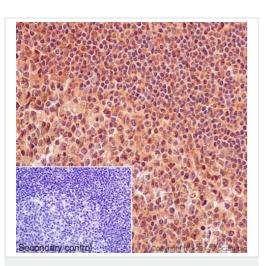
Lysates/proteins at 20 µg per lane.

#### Secondary

All lanes : HRP goat anti-rabbit (H+L) at 1/1000 dilution

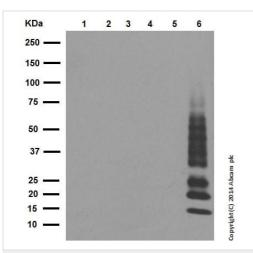
Predicted band size: 77 kDa Observed band size: 16-300 kDa

Blocking buffer: 5% NFDM/TBST Dilution buffer: 5% NFDM/TBST



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Ubiquitin (linkage-specific K63) antibody [EPR8590-448] (ab179434)

Immunohistochemical staining of paraffin embedded human tonsil with purified ab179434 at a dilution of 1/500. The secondary antibody used is a HRP conjugated goat anti-rabbit (**ab97051**, 1/500). The sample is counter-stained with hematoxylin. Antigen retrieval was perfomed using Tris-EDTA buffer, pH 9.0. PBS was used instead of the primary antibody as the negative control, and is shown in the inset.



All lanes : Anti-Ubiquitin (linkage-specific K63) antibody [EPR8590-448] (ab179434) at 1/5000 dilution (purified)

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

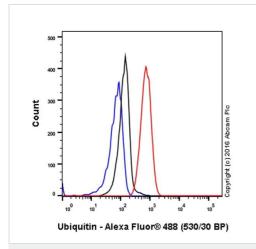
Lysates/proteins at 20 µg per lane.

#### Secondary

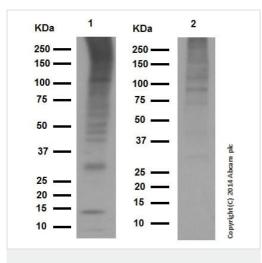
All lanes : HRP goat anti-rabbit (H+L) at 1/1000 dilution

Predicted band size: 77 kDa Observed band size: 16-70 kDa

Blocking buffer: 5% NFDM/TBST Dilution buffer: 5% NFDM/TBST



Flow Cytometry (Intracellular) - Anti-Ubiquitin (linkage-specific K63) antibody [EPR8590-448] (ab179434) Intracellular Flow Cytometry analysis of HeLa (human cervix adenocarcinoma) cells labelling Ubiquitin (linkage-specific K63) with purified ab179434 at 1/210 (red). Cells were fixed with 4% paraformaldehyde and permeabilised with 90% methanol. An Alexa Fluor<sup>®</sup>488-conjugated goat anti-rabbit IgG (1/2000) was used as the secondary antibody. Black - Isotype control, rabbit monoclonal IgG. Blue - Unlabelled control, cells without incubation with primary and secondary antibodies.



All lanes : Anti-Ubiquitin (linkage-specific K63) antibody [EPR8590-448] (ab179434) at 1/1000 dilution (purified)

Lane 1 : Mouse brain tissue lysate Lane 2 : Rat brain tissue lysate

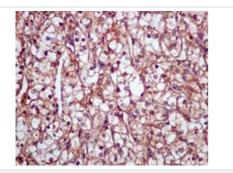
Lysates/proteins at 20 µg per lane.

#### Secondary

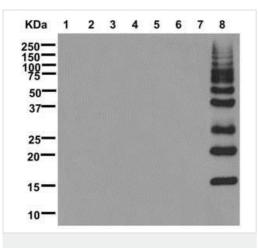
All lanes : HRP goat anti-rabbit (H+L) at 1/1000 dilution

Predicted band size: 77 kDa Observed band size: 16-300 kDa

Blocking buffer: 5% NFDM/TBST Dilution buffer: 5% NFDM/TBST



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Ubiquitin (linkage-specific K63) antibody [EPR8590-448] (ab179434) Immunohistochemical analysis of paraffin-embedded human kidney carcinoma tissue labeling K63-linkage specific polyubiquitin with unpurified ab179434 at 1/100 dilution.



**All lanes :** Anti-Ubiquitin (linkage-specific K63) antibody [EPR8590-448] (ab179434) at 1/1000 dilution (unpurified)

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

Lysates/proteins at 0.02 µg per lane.

Predicted band size: 77 kDa



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