

Product datasheet

Anti-Ubiquitin antibody [EPR8830] ab134953

Recombinant RabMAb

★★★★★ 1 Abreviews 11 References 13 Images

Overview

Product name	Anti-Ubiquitin antibody [EPR8830]
Description	Rabbit monoclonal [EPR8830] to Ubiquitin
Host species	Rabbit
Tested applications	Suitable for: WB, IHC-P, ICC/IF, Flow Cyt
Species reactivity	Reacts with: Mouse, Rat, Human
Immunogen	Synthetic peptide within Human Ubiquitin aa 1-100. The exact sequence is proprietary. (Peptide available as ab220157)
Positive control	293T cell lysate; HepG2 whole cell lysate (ab7900); HeLa whole cell lysate (ab150035); Paraffin embedded Human breast carcinoma tissue, JAR cells; Mouse liver tissue; Human bladder carcinoma tissue; Rat liver tissue; Human bladder carcinoma; Rat liver; Mouse liver.
General notes	A trial size is available to purchase for this antibody.

Our RabMAb[®] technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to [RabMAb[®] patents](#).

We are constantly working hard to ensure we provide our customers with best in class antibodies. As a result of this work we are pleased to now offer this antibody in purified format. We are in the process of updating our datasheets. The purified format is designated 'PUR' on our product labels. If you have any questions regarding this update, please contact our Scientific Support team.

This product is a [recombinant rabbit monoclonal antibody](#).

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. Stable for 12 months at -20°C.
Storage buffer	Preservative: 0.01% Sodium azide Constituents: 40% Glycerol, 0.05% BSA, 59% PBS
Purity	Protein A purified

Clonality	Monoclonal
Clone number	EPR8830
Isotype	IgG

Applications

Our [Abpromise guarantee](#) covers the use of **ab134953** 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
WB		1/1000 - 1/10000. Predicted molecular weight: 8 kDa. Can be blocked with Ubiquitin peptide (ab220157) . Monoubiquitin molecular weight
IHC-P	★★★★★	1/800. See IHC antigen retrieval protocols . For unpurified use at 1/250 - 1/500.
ICC/IF		1/100. For unpurified use at 1/250 - 1/500.
Flow Cyt		1/70. For unpurified use at 1/100 - 1/1000. ab172730 - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.

Target

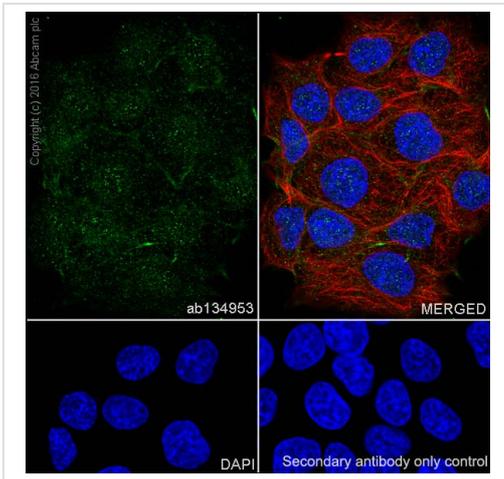
Relevance

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

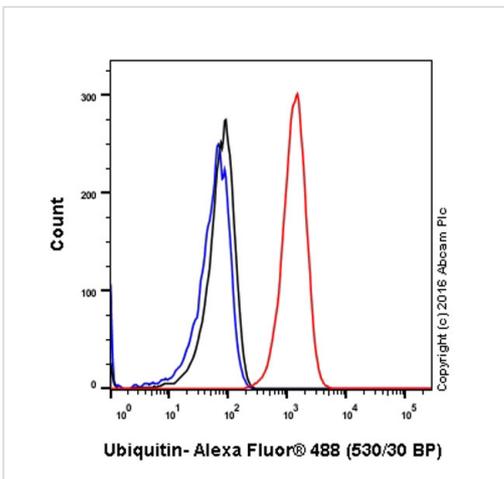
Cell Membrane, Cytoplasmic and Nuclear

Images



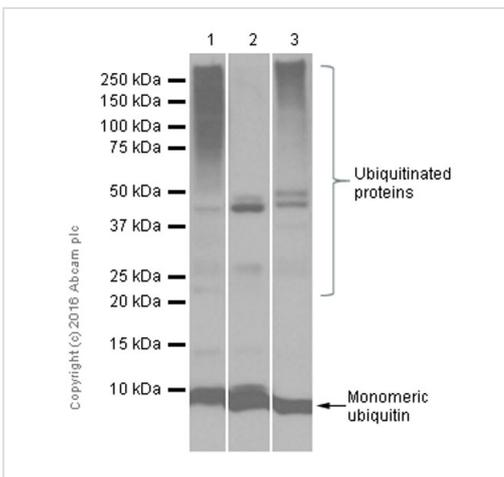
Immunocytochemistry/ Immunofluorescence - Anti-Ubiquitin antibody [EPR8830] (ab134953)

Immunocytochemistry/ Immunofluorescence analysis of JAR (Human placenta choriocarcinoma cell line) cells labeling Ubiquitin with Purified ab134953 at 1:100 dilution (7.2µg/ml). Cells were fixed in 4% Paraformaldehyde and permeabilized with 0.1% tritonX-100. Cells were counterstained with Ab195889 Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) 1:200 (2.5 µg/ml). ab150077 Goat anti rabbit IgG(Alexa Fluor® 488) was used as the secondary antibody at 1:1000 dilution. DAPI nuclear counterstain. PBS instead of the primary antibody was used as the secondary antibody only control.



Flow Cytometry - Anti-Ubiquitin antibody [EPR8830] (ab134953)

Flow Cytometry analysis of JAR (Human placenta choriocarcinoma cell line) cells labeling Ubiquitin with purified ab134953 at 1:70 dilution (10 ug/ml) (red). Cells were fixed with 4% Paraformaldehyde. A Goat anti rabbit IgG (Alexa Fluor® 488) secondary antibody was used at 1:2000 dilution. Isotype control - Rabbit monoclonal IgG (Black). Unlabeled control - Cell without incubation with primary antibody and secondary antibody (Blue).



Western blot - Anti-Ubiquitin antibody [EPR8830] (ab134953)

All lanes : Anti-Ubiquitin antibody [EPR8830] (ab134953) at 0.7 µg/ml (Purified)

Lane 1 : Rat brain lysate

Lane 2 : Mouse kidney lysate

Lane 3 : Rat kidney 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: 8 kDa

Observed band size: 8 kDa

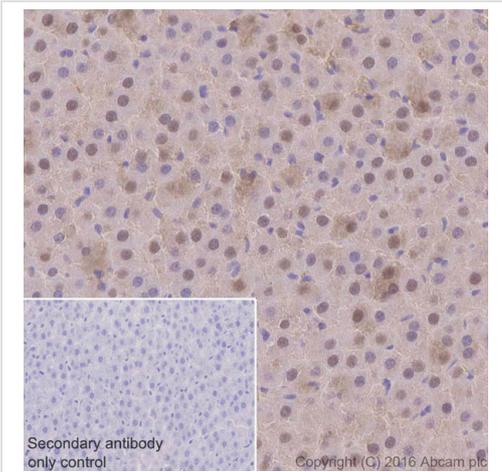
Blocking and diluting buffer: 5% NFDm/TBST



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Ubiquitin antibody
[EPR8830] (ab134953)

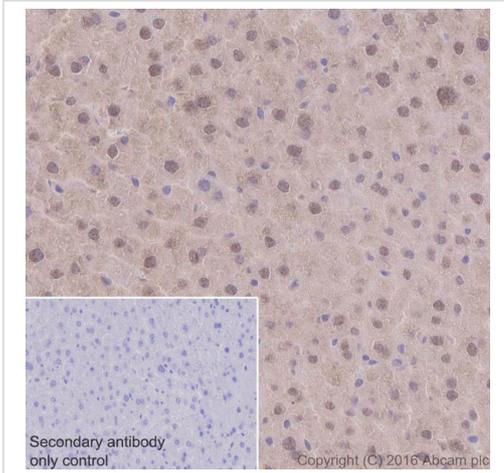
This image is courtesy of an anonymous Abreview

ab134953 staining Ubiquitin in Mouse embryo tissue sections by Immunohistochemistry (IHC-P - paraformaldehyde-fixed, paraffin-embedded sections). Tissue was fixed with formaldehyde and blocked with 1% FBS/BSA for 1 hour at room temperature; antigen retrieval was by heat mediation in citrate buffer, pH6. Samples were incubated with primary antibody (1/100 in 1% FBS/BSA) for 16 hours at 4°C. An undiluted HRP-conjugated Goat anti-rabbit IgG polyclonal was used as the secondary antibody.



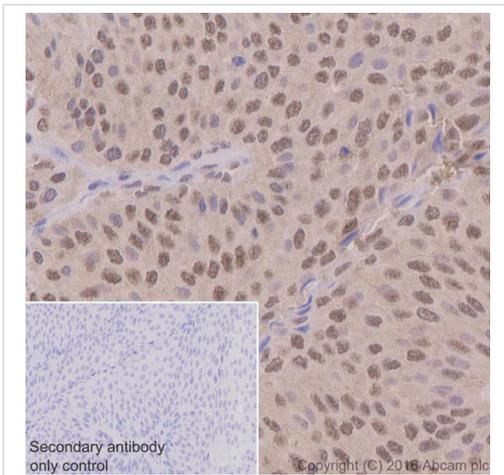
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Ubiquitin antibody
[EPR8830] (ab134953)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of Rat liver tissue sections labeling Ubiquitin with Purified ab134953 at 1:800 dilution (0.9 µg/ml). Heat mediated antigen retrieval was performed using Perform heat mediated antigen retrieval using EDTA Buffer, PH9. Tissue was counterstained with Hematoxylin. [ab97051](#) Goat Anti-Rabbit IgG H&L (HRP) secondary antibody was used at 1:500 dilution. PBS instead of the primary antibody was used as the negative control.



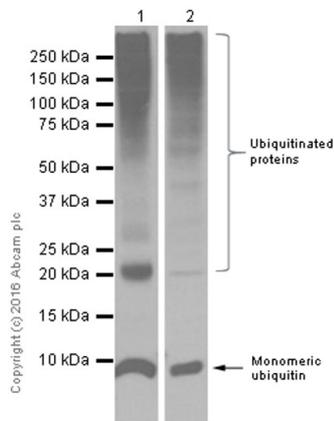
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of Mouse liver tissue sections labeling Ubiquitin with Purified ab134953 at 1:800 dilution (0.9 µg/ml). Heat mediated antigen retrieval was performed using Perform heat mediated antigen retrieval using EDTA Buffer, PH9. Tissue was counterstained with Hematoxylin. [ab97051](#) Goat Anti-Rabbit IgG H&L (HRP) secondary antibody was used at 1:500 dilution. PBS instead of the primary antibody was used as the negative control.

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Ubiquitin antibody [EPR8830] (ab134953)



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of Human bladder carcinoma tissue sections labeling Ubiquitin with Purified ab134953 at 1:800 dilution (0.9 µg/ml). Heat mediated antigen retrieval was performed using Perform heat mediated antigen retrieval using EDTA Buffer, PH9. Tissue was counterstained with Hematoxylin. [ab97051](#) Goat Anti-Rabbit IgG H&L (HRP) secondary antibody was used at 1:500 dilution. PBS instead of the primary antibody was used as the negative control.

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Ubiquitin antibody [EPR8830] (ab134953)



Western blot - Anti-Ubiquitin antibody [EPR8830] (ab134953)

All lanes : Anti-Ubiquitin antibody [EPR8830] (ab134953) at 0.1 µg/ml (Purified)

Lane 1 : JAR (Human placenta choriocarcinoma epithelial cell) whole cell lysate

Lane 2 : 293 (Human embryonic kidney epithelial cell) whole cell lysate

Lysates/proteins at 20 µg per lane.

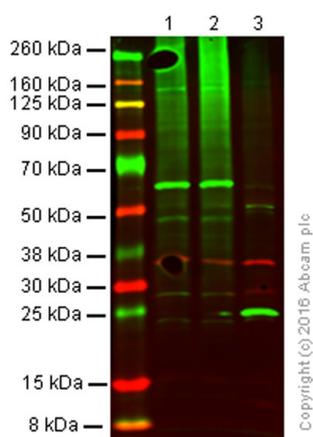
Secondary

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

Predicted band size: 8 kDa

Observed band size: 8 kDa

Blocking and diluting buffer: 5% NFDm/TBST



Western blot - Anti-Ubiquitin antibody [EPR8830] (ab134953)

All lanes : Anti-Ubiquitin antibody [EPR8830] (ab134953) at 1/200 dilution

Lane 1 : MCF-7 Whole cell lysate

Lane 2 : MCF-7 Whole cell lysate + M132 (50 µM 90 min)

Lane 3 : Mouse Brain

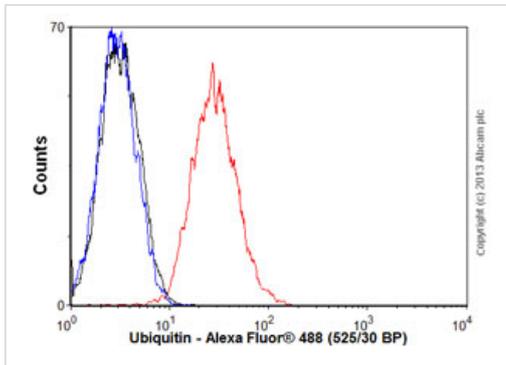
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 8 kDa

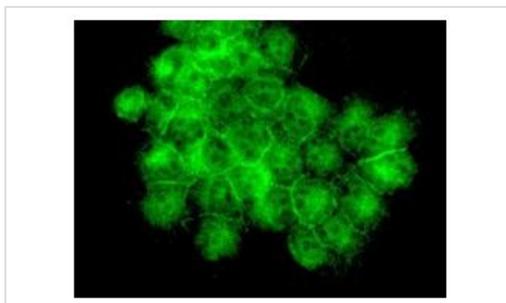
This blot was produced using a 4-12% Bis-tris gel under the MES buffer system. The gel was run at 200V for 35 minutes before being transferred onto a nitrocellulose membrane at 30V for 70 minutes. ab134953 and ab8245 (loading control to GAPDH) were diluted

1/200 and 1/10 000 respectively and incubated overnight at 4°C. Blots were developed with goat anti-rabbit IgG (H + L) and goat anti-mouse IgG (H + L) secondary antibodies at 1/10 000 dilution for 1 h at room temperature before imaging using the Licor Odyssey CLx.



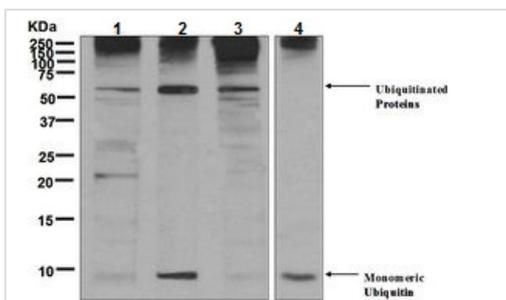
Flow Cytometry - Anti-Ubiquitin antibody [EPR8830] (ab134953)

Overlay histogram showing HepG2 cells stained with ab134953 (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 (ab134953, 1/1000 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) (0.1µg/1x10⁶ 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 antibody gave a positive signal in HepG2 cells fixed with 4% paraformaldehyde (10 min)/permeabilized with 0.1% PBS-Tween for 20 min used under the same conditions.



Immunocytochemistry/ Immunofluorescence - Anti-Ubiquitin antibody [EPR8830] (ab134953)

Immunofluorescent staining of JAR cells labelling Ubiquitin using ab134953 at 1/250 dilution



Western blot - Anti-Ubiquitin antibody [EPR8830] (ab134953)

All lanes : Anti-Ubiquitin antibody [EPR8830] (ab134953) at 1/1000 dilution

- Lane 1 :** 293T cell lysate
- Lane 2 :** HepG2 cell lysate
- Lane 3 :** HeLa cell lysate
- Lane 4 :** JAR cell lysate

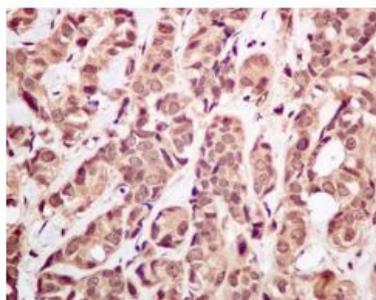
Lysates/proteins at 10 µg per lane.

Secondary

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

Predicted band size: 8 kDa

Note: Ubiquitin exists as monomeric ubiquitin (8 kDa) or attached to other proteins.



Immunohistochemical analysis of paraffin embedded Human breast carcinoma tissue labelling Ubiquitin with ab134953 at 1/250 dilution

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Ubiquitin antibody [EPR8830] (ab134953)

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