abcam

Product datasheet

Anti-Ubiquitin antibody [EPR8830] ab134953

יעלאעבע RabMAb

★★★★★ 3 Abreviews 74 References 画像数 14

製品の概要

製品名 Anti-Ubiquitin antibody [EPR8830]

製品の詳細 Rabbit monoclonal [EPR8830] to Ubiquitin

由来種 Rabbit

アプリケーション 適用あり: WB, IHC-P, ICC/IF, Flow Cyt (Intra)

種交差性 交差種: Mouse, Rat, Human

免疫原 Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.

ポジティブ・コントロール ICC: JAR cells. Flow Cyt (intra): JAR and HepG2 cells. WB: Rat brain and kidney lysate. Mouse

> kidney lysate. JAR, MCF7, HeLa, HEK-293T and HEK-293 whole cell lysate. IHC-P: Mouse embryo tissue. Rat and mouse liver tissue. Human bladder carcinoma and breast carcinoma

tissue.

特記事項 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**.

製品の特性

製品の状態 Liquid

保存方法 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.

バッファー pH: 7.2

Preservative: 0.01% Sodium azide

Constituents: 40% Glycerol, 0.05% BSA, 59% PBS

精製度 Protein A purified

ポリ/モノ モノクローナル クローン名 **EPR8830**

アプリケーション

The Abpromise guarantee <u>Abpromise保証は、</u>次のテスト済みアプリケーションにおけるab134953の使用に適用されます アプリケーションノートには、推奨の開始希釈率がありますが、適切な希釈率につきましてはご検討ください。

アプリケーション	Abreviews	特記事項
WB	★★★☆☆ (1)	1/1000 - 1/10000. Predicted molecular weight: 8 kDa. Monoubiquitin molecular weight
IHC-P	****(1)	1/800. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol. 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 (Intra)		1/70. For unpurified use at 1/100 - 1/1000. <u>ab172730</u> - Rabbit monoclonal lgG, is suitable for use as an isotype control with this antibody.

ターゲット情報

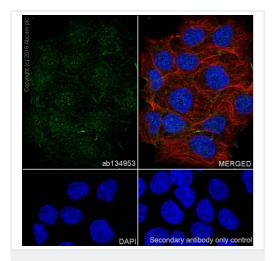
関連性

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-11linked 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 ubiquitinlike domains.

細胞内局在

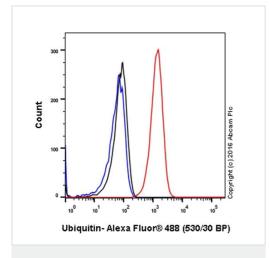
Cell Membrane, Cytoplasmic and Nuclear

画像



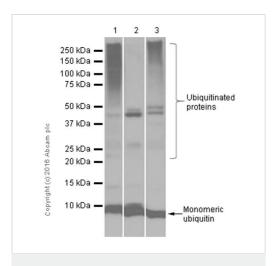
Immunocytochemistry/ Immunofluorescence - Anti-Ubiquitin antibody [EPR8830] (ab134953)

Immunocytochemistry/ Immunofluorescence analysis of JAR (Human placenta choriocarcinoma cell line) cells labeling Ubiquitin with Purified ab134953 at 1:100 dilution (7.2 μ g/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 μ g/ml). **ab150077** Goat anti rabbit lgG(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 (Intracellular) - Anti-Ubiquitin antibody [EPR8830] (ab134953)

Intracellular 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 lgG (Alexa Fluor® 488) secondary antibody was used at 1/2000 dilution. Isotype control - Rabbit monoclonal lgG (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.

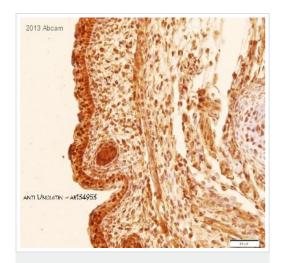
Secondary

All lanes : Goat Anti-Rabbit lgG H&L (HRP) (<u>ab97051</u>) at 1/20000

dilution

Predicted band size: 8 kDa **Observed band size:** 8 kDa

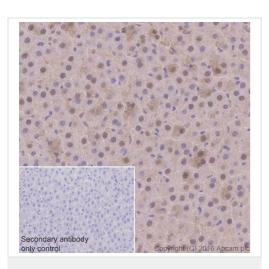




Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-Ubiquitin antibody
[EPR8830] (ab134953)

This image is courtesy of an anonymous Abreview

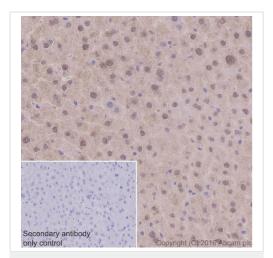
ab134953 staining Ubiquitin in Mouse embryo tissue sections by Immunohistochemistry (IHC-P - paraformaldehyde-fixed, paraffinembedded sections). Tissue was fixed with formaldehyde and blocked with 1% FBS/BSA for 1 hour at room temperature; antigen retrieval was by heat mediation in citrate buffer, pH6. 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 paraffinembedded 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 μ g/ml). Heat mediated antigen retrieval was performed using EDTA Buffer, pH 9.0. Tissue was counterstained with Hematoxylin. <u>ab97051</u> Goat Anti-Rabbit lgG H&L (HRP)

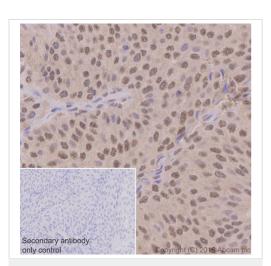
secondary antibody was used at 1:500 dilution. PBS instead of the primary antibody was used as the negative control.



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

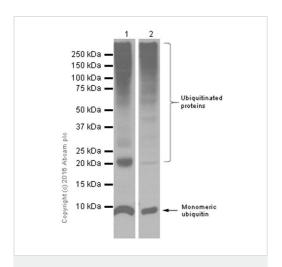
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of Mouse liver tissue sections labeling Ubiquitin with Purified ab134953 at 1:800 dilution (0.9 µg/ml). Heat mediated antigen retrieval was performed using EDTA Buffer, pH 9.0. Tissue was counterstained with Hematoxylin. ab97051 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 paraffinembedded 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 EDTA Buffer, pH 9.0. Tissue was counterstained with Hematoxylin. ab97051 Goat Anti-Rabbit lgG H&L (HRP) secondary antibody was used at 1:500 dilution. PBS instead of the primary antibody was used as the negative control.



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

All lanes : Anti-Ubiquitin antibody [EPR8830] (ab134953) at 0.1 μ g/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.

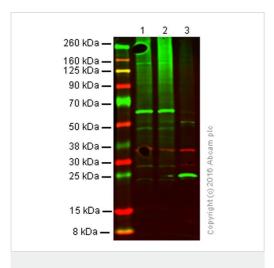
Secondary

All lanes : Goat Anti-Rabbit lgG H&L (HRP) (<u>ab97051</u>) 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 + MG-132 (50 uM 90 min)

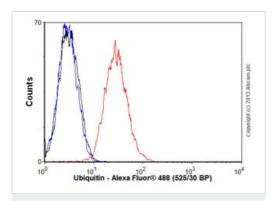
Lane 3: Mouse Brain

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

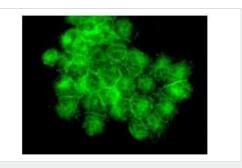
Predicted band size: 8 kDa

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.



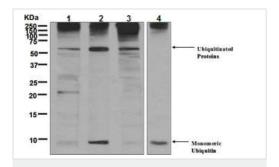
Flow Cytometry (Intracellular) - Anti-Ubiquitin antibody [EPR8830] (ab134953)

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 lgG (H&L) (ab150077) at 1/2000 dilution for 30 min at 22°C. Isotype control antibody (black line) was rabbit lgG (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

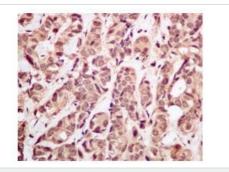
Lysates/proteins at 10 µg per lane.

Secondary

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

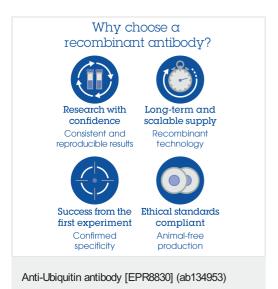
Predicted band size: 8 kDa

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



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

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



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