abcam

Product datasheet

Anti-UBE3A antibody [EPR23077-14] ab272168

Recombinant RabMAb

11 Images

Overview

Product name	Anti-UBE3A antibody [EPR23077-14]
Description	Rabbit monoclonal [EPR23077-14] to UBE3A
Host species	Rabbit
Tested applications	**Suitable for:** Indirect ELISA, IP, WB, ICC/IF, Flow Cyt (Intra) **Unsuitable for:** IHC-P
Species reactivity	**Reacts with:** Mouse, Rat, Human
Immunogen	Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: HeLa, K562, HepG2,A549, HEK-293T, PC-12, PC-12 (treated with 10 uM MG-132 for 4 hours), RAW 264.7 and RAW 264.7 (treated with 10 uM MG-132 for 4 hours) whole cell lysates; Mouse spleen tissue lysate;, Rat brain tissue lysate. ICC/IF: HeLa and RAW 264.7 cells. Flow Cyt (intra): HeLa and RAW 264.7 cells. IP: K562 and RAW 264.7 whole cell lysates.
General notes	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. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C long term. Avoid freeze / thaw cycle.
Storage buffer	pH: 7.2 Preservative: 0.01% Sodium azide Constituents: PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
Purity	Protein A purified
Clonality	Monoclonal

Clone number	EPR23077-14
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab272168 in the following tested applications.

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

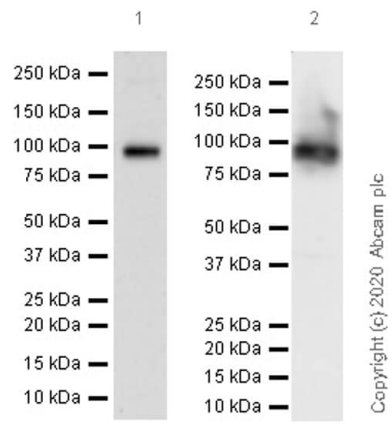
Application	Abreviews	Notes
Indirect ELISA		Use a concentration of 1 µg/ml.
IP		1/30.
WB		1/1000. Detects a band of approximately 100, 37 kDa (predicted molecular weight: 100 kDa).
ICC/IF		1/100.
Flow Cyt (Intra)		1/50.

Application notes Is unsuitable for IHC-P.

Target

Function	E3 ubiquitin-protein ligase which accepts ubiquitin from an E2 ubiquitin-conjugating enzyme in the form of a thioester and transfers it to its substrates. Several substrates have been identified including the RAD23A and RAD23B, MCM7 (which is involved in DNA replication), annexin A1, the PML tumor suppressor, and the cell cycle regulator CDKN1B. Catalyzes the high-risk human papilloma virus E6-mediated ubiquitination of p53/TP53, contributing to the neoplastic progression of cells infected by these viruses. Additionally, may function as a cellular quality control ubiquitin ligase by helping the degradation of the cytoplasmic misfolded proteins. Finally, UBE3A also promotes its own degradation in vivo. Plays an important role in the regulation of the circadian clock: involved in the ubiquitination of the core clock component ARNTL/BMAL1, leading to its proteasomal degradation (PubMed:24728990).
Pathway	Protein modification; protein ubiquitination.
Involvement in disease	Angelman syndrome
Sequence similarities	Contains 1 HECT (E6AP-type E3 ubiquitin-protein ligase) domain.
Post-translational modifications	Phosphorylation at Tyr-659 by ABL1 impairs E3 ligase activity and protects p53/TP53 from degradation in (HPV)-infected cells.
Cellular localization	Nucleus. Cytoplasm.

Images



Western blot - Anti-UBE3A antibody [EPR23077-14] (ab272168)

All lanes : Anti-UBE3A antibody [EPR23077-14] (ab272168) at 1/1000 dilution

Lane 1 : HeLa (human cervix adenocarcinoma epithelial cell) treated with 10 uM MG-132 for 24 hours, whole cell lysate
Lane 2 : HEK-293T (human embryonic kidney epithelial cell) whole cell lysate

Lysates/proteins at 20 µg per lane.

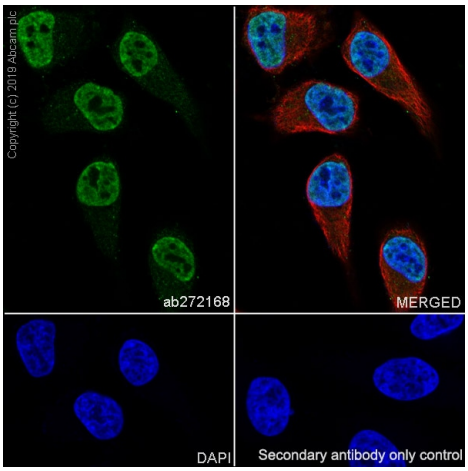
Secondary
All lanes : Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated (**ab97051**) at 1/100000 dilution

Predicted band size: 100 kDa
Observed band size: 100 kDa

Blocking and diluting buffer and concentration: 5% NFDM/TBST.

Fresh lysate was used in lane 2.

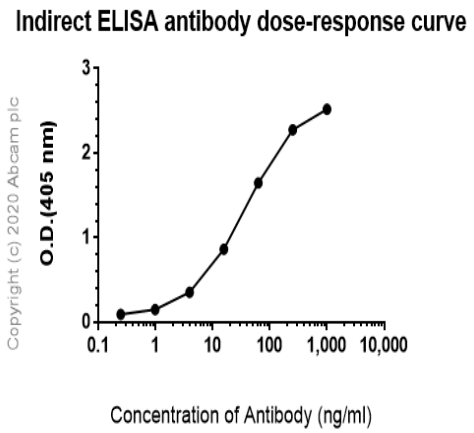
Exposure time: Lane 1: 3 minutes; Lane 2: 48 seconds.



Immunocytochemistry/ Immunofluorescence - Anti-UBE3A antibody [EPR23077-14] (ab272168)

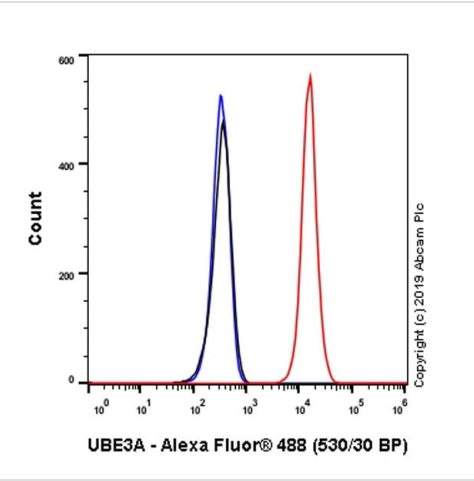
Immunofluorescent analysis of 4% Paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized HeLa cells labelling UBE3A with ab272168 at 1/100 dilution, followed by **ab150077** Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) antibody at 1/1000 dilution (Green). Confocal image showing nuclear and weak cytoplasmic staining in HeLa cell line **ab195889**. Anti-alpha Tubulin antibody (Alexa Fluor® 594) was used to counterstain tubulin at 1/200 dilution (Red). The Nuclear counterstain was DAPI (Blue).

Secondary antibody only control: Secondary antibody is **ab150077** Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) at 1000 dilution.



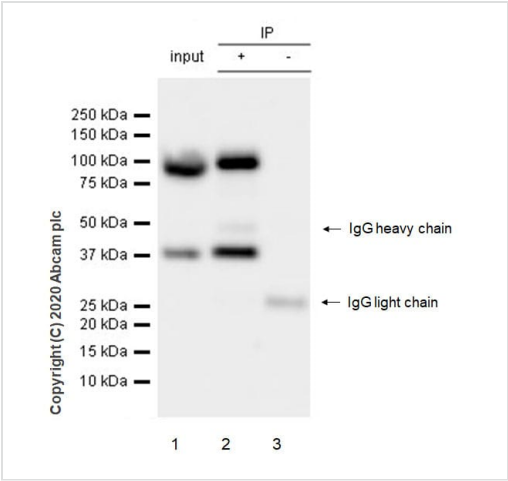
Indirect ELISA - Anti-UBE3A antibody [EPR23077-14] (ab272168)

Indirect ELISA using ab272168 at varying antibody concentrations (1000-0 ng/ml) and Human UBE3A antigen at 1000 ng/ml. Alkaline Phosphatase-conjugated AffiniPure Goat Anti-Rabbit IgG (H+L) at 1/2500 dilution was used as a secondary antibody.



Flow Cytometry (Intracellular) - Anti-UBE3A antibody [EPR23077-14] (ab272168)

Intracellular flow cytometric analysis of 4% paraformaldehyde fixed, 90% methanol-permeabilized HeLa (Human cervix adenocarcinoma epithelial cell) cells labelling UBE3A with ab272168 at 1/500 dilution (Red) compared with a Rabbit monoclonal IgG (**ab172730**) (Black) isotype control and an unlabelled control (cells without incubation with primary antibody and secondary antibody) (Blue). A Goat anti rabbit IgG (Alexa Fluor® 488, **ab150077**) at 1/2000 dilution was used as the secondary antibody.



Immunoprecipitation - Anti-UBE3A antibody [EPR23077-14] (ab272168)

UBE3A was immunoprecipitated from 0.35 mg K-562 (human chronic myelogenous leukemia lymphoblast) whole cell lysate with ab272168 at 1/30 dilution (2ug in 0.35mg lysates). Western blot was performed on the immunoprecipitate using ab272168 at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP) (**ab131366**) was used at 1/5000 dilution.

Lane 1: K-562 (human chronic myelogenous leukemia lymphoblast) whole cell lysate 10 ug

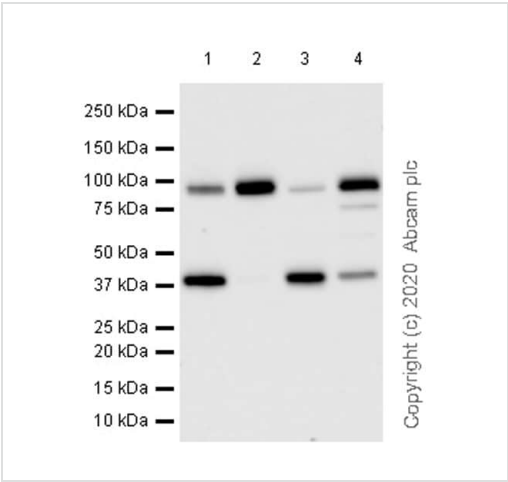
Lane 2: ab272168 IP in K-562 whole cell lysate

Lane 3: Rabbit monoclonal IgG (**ab172730**) instead of ab272168 in K-562 whole cell lysate

Blocking and dilution buffer and concentration: 5% NFDM/TBST.

Exposure time: 15 seconds

A 37 kDa degraded band is observed.



Western blot - Anti-UBE3A antibody [EPR23077-14] (ab272168)

All lanes : Anti-UBE3A antibody [EPR23077-14] (ab272168) at 1/1000 dilution

Lane 1 : PC-12 (rat adrenal gland pheochromocytoma) whole cell lysate
Lane 2 : PC-12 treated with 10 µM MG-132 for 4 hours, whole cell lysate
Lane 3 : RAW 264.7 (mouse abelson murine leukemia virus-induced tumor macrophage) whole cell lysate
Lane 4 : RAW 264.7 treated with 10 µM MG-132 for 4 hours, whole cell lysate

Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (**ab97051**) at 1/100000 dilution

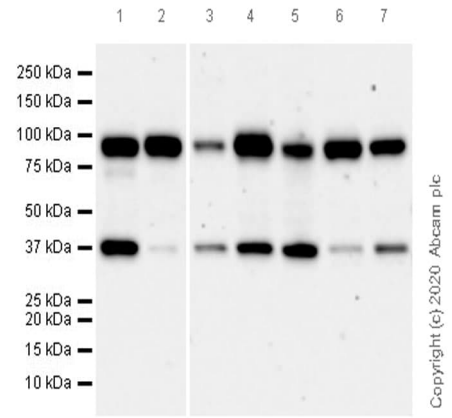
Predicted band size: 100 kDa
Observed band size: 100,37 kDa

Blocking and dilution buffer: 5% NFDM/TBST.

Exposure time: 3 minutes.

The 37-kDa band might be a degraded fragment (not verified), which can be inhibited/reduced by MG-132 treatment (lanes 2 and

4).



Western blot - Anti-UBE3A antibody [EPR23077-14] (ab272168)

All lanes : Anti-UBE3A antibody [EPR23077-14] (ab272168) at 1/1000 dilution

Lane 1 : Mouse spleen tissue lysate
Lane 2 : Rat brain tissue lysate
Lane 3 : HeLa (human cervix adenocarcinoma epithelial cell) whole cell lysate
Lane 4 : K-562 (human chronic myelogenous leukemia lymphoblast) whole cell lysate
Lane 5 : HepG2 (human hepatocellular carcinoma epithelial cell) whole cell lysate
Lane 6 : HEK-293T (human embryonic kidney epithelial cell) whole cell lysate
Lane 7 : A549 (human lung carcinoma epithelial cell) whole cell lysate

Lysates/proteins at 20 µg per lane.

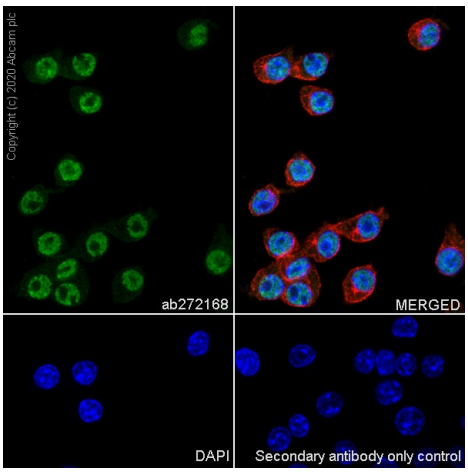
Secondary
All lanes : Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated (**ab97051**) at 1/100000 dilution

Predicted band size: 100 kDa
Observed band size: 100,37 kDa

Exposure time: 3 minutes

Blocking and diluting buffer and concentration: 5% NFDM/TBST.

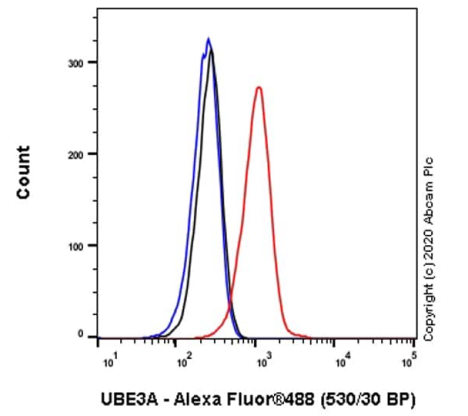
The 37 kDa band might be a degraded band (not verified). MG132 treatment or freshly made lysates can decrease the degradation.



Immunocytochemistry/ Immunofluorescence - Anti-UBE3A antibody [EPR23077-14] (ab272168)

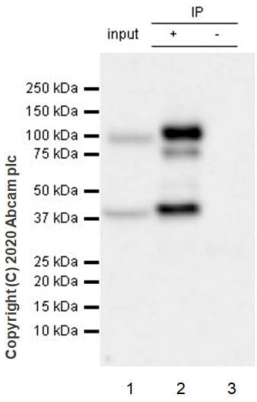
Immunofluorescent analysis of 4% Paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized RAW 264.7 cells labelling UBE3A with ab272168 at 1/100 dilution, followed by **ab150077** Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) antibody at 1/1000 dilution (Green). Confocal image showing nuclear and weak cytoplasmic staining in RAW 264.7 cell line. **ab195889** Anti-alpha Tubulin antibody (Alexa Fluor® 594) was used to counterstain tubulin at 1/200 dilution (Red). The Nuclear counterstain was DAPI (Blue).

Secondary antibody only control: Secondary antibody is **ab150077** Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) at 1000 dilution.



Flow Cytometry (Intracellular) - Anti-UBE3A antibody [EPR23077-14] (ab272168)

Intracellular flow cytometric analysis of 4% paraformaldehyde fixed, 90% methanol-permeabilized RAW 264.7 (mouse abelson murine leukemia virus-induced tumor macrophage) cells labelling UBE3A with ab272168 at 1/50 dilution (Red) compared with a Rabbit monoclonal IgG (**ab172730**) (Black) isotype control and an unlabelled control (cells without incubation with primary antibody and secondary antibody) (Blue). A Goat anti rabbit IgG (Alexa Fluor® 488, **ab150077**) at 1/2000 dilution was used as the secondary antibody.



Immunoprecipitation - Anti-UBE3A antibody [EPR23077-14] (ab272168)

UBE3A was immunoprecipitated from 0.35 mg RAW 264.7 (mouse abelson murine leukemia virus-induced tumor macrophage) (treated with 10 µM MG-132 for 4 hours) whole cell lysate with ab272168 at 1/30 dilution (2ug in 0.35mg lysates). Western blot was performed on the immunoprecipitate using ab272168 at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP) (**ab131366**) was used at 1/5000 dilution.

Lane 1: RAW 264.7 (mouse abelson murine leukemia virus-induced tumor macrophage) treated with 10 µM MG-132 for 4 hours, whole cell lysate 10 ug

Lane 2: ab272168 IP in RAW 264.7 treated with 10 µM MG-132 for 4 hours, whole cell lysate

Lane 3: Rabbit monoclonal IgG (**ab172730**) instead of ab272168 in RAW 264.7 treated with 10 µM MG-132 for 4 hours, whole cell lysate

Blocking and dilution buffer and concentration: 5% NFDM/TBST.

Exposure time: 10 seconds

A 37 kDa degraded band is observed.

Anti-UBE3A antibody [EPR23077-14] (ab272168)

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