abcam

Product datasheet

Anti-RING2 / RING1B / RNF2 antibody [EPR12245] ab181140

KO VALIDATED Recombinant RabMAb

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Overview

Product name	Anti-RING2 / RING1B / RNF2 antibody [EPR12245]
Description	Rabbit monoclonal [EPR12245] to RING2 / RING1B / RNF2
Host species	Rabbit
Tested applications	**Suitable for:** Flow Cyt (Intra), WB, IHC-P, ICC/IF
Species reactivity	**Reacts with:** Mouse, Human **Predicted to work with:** Rat
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	HeLa, 293, Jurkat and HepG2 whole cell lysate (**ab7900**); Human placenta tissue; HepG2 cells.
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	Preservative: 0.01% Sodium azide Constituents: 40% Glycerol (glycerin, glycerine), 59% PBS, 0.05% BSA
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPR12245

Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab181140 in the following tested applications.

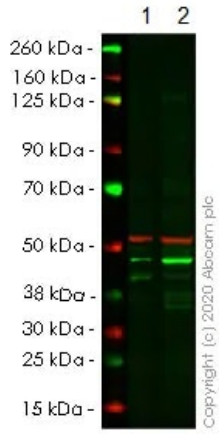
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
Flow Cyt (Intra)		Use at an assay dependent concentration.
WB		1/1000 - 1/10000. Detects a band of approximately 41 kDa (predicted molecular weight: 38 kDa).
IHC-P	★★★★☆ (3)	1/250 - 1/500. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
ICC/IF		1/100 - 1/250.

Target

Function	E3 ubiquitin-protein ligase that mediates monoubiquitination of 'Lys-119' of histone H2A, thereby playing a central role in histone code and gene regulation. H2A 'Lys-119' ubiquitination gives a specific tag for epigenetic transcriptional repression and participates in X chromosome inactivation of female mammals. May be involved in the initiation of both imprinted and random X inactivation. Essential component of the Polycomb group (PcG) multiprotein PRC1 complex, a complex required to maintain the transcriptionally repressive state of many genes, including Hox genes, throughout development. PcG PRC1 complex act via chromatin remodeling and modification of histones, rendering chromatin heritably changed in its expressibility. E3 ubiquitin-protein ligase activity is enhanced by BMI1/PCGF4. Acts as the main E3 ubiquitin ligase on histone H2A of the PRC1 complex, while RING1 may rather act as a modulator of RNF2/RING2 activity.
Pathway	Protein modification; protein ubiquitination.
Sequence similarities	Contains 1 RING-type zinc finger.
Post-translational modifications	Polyubiquitinated in the presence of UBE2D3 (in vitro). Monoubiquitinated, by auto-ubiquitination.
Cellular localization	Nucleus. Chromosome. Enriched on inactive X chromosome (Xi) in female trophoblast stem (TS) cells as well as differentiating embryonic stem (ES) cells. The enrichment on Xi is transient during TS and ES cell differentiation. The association with Xi is mitotically stable in non-differentiated TS cells.

Images



Western blot - Anti-RING2 / RING1B / RNF2 antibody [EPR12245] (ab181140)

All lanes : Anti-RING2 / RING1B / RNF2 antibody [EPR12245] (ab181140) at 1/1000 dilution

Lane 1 : Wild-type HeLa cell lysate
Lane 2 : RNF2 CRISPR/Cas9 edited HeLa cell lysate

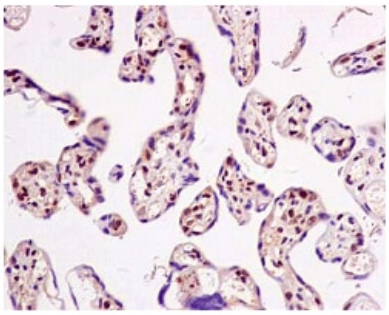
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 38 kDa
Observed band size: 42 kDa

Lanes 1- 2: Merged signal (red and green). Green - ab181140 observed at 42 kDa. Red - Anti-alpha Tubulin antibody [DM1A] - Loading Control (**ab7291**) observed at 50 kDa.

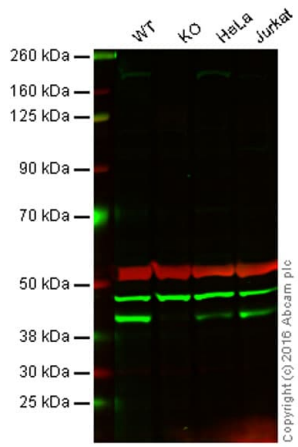
ab181140 was shown to react with RING2 / RING1B / RNF2 in wild-type HeLa cells in western blot. The band observed in CRISPR/Cas9 edited cell line **ab264845** (CRISPR/Cas9 edited cell lysate **ab257640**) lane below 42kDa may represent truncated forms and cleaved fragments. This has not been investigated further. Wild-type HeLa and RNF2 CRISPR/Cas9 edited HeLa cell lysates were subjected to SDS-PAGE. Membrane was blocked for 1 hour at room temperature in 0.1% TBST with 3% non-fat dried milk. ab181140 and Anti-alpha Tubulin antibody [DM1A] - Loading Control (**ab7291**) were incubated overnight at 4°C at a 1 in 1000 dilution and a 1 in 20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye®800CW) preadsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye®680RD) preadsorbed (**ab216776**) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-RING2 / RING1B / RNF2 antibody [EPR12245] (ab181140)

Immunohistochemical analysis of paraffin-embedded Human placenta tissue labeling RING2 / RING1B / RNF2 with ab181140 at 1/500 dilution followed by pre-diluted HRP-conjugated secondary antibody and counter-stained with Hematoxylin.

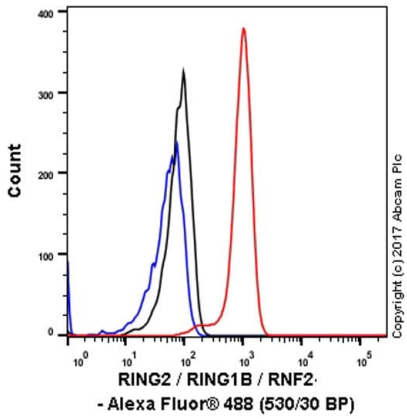
Perform heat mediated antigen retrieval with EDTA buffer pH 9 before commencing with IHC staining protocol.



Western blot - Anti-RING2 / RING1B / RNF2 antibody [EPR12245] (ab181140)

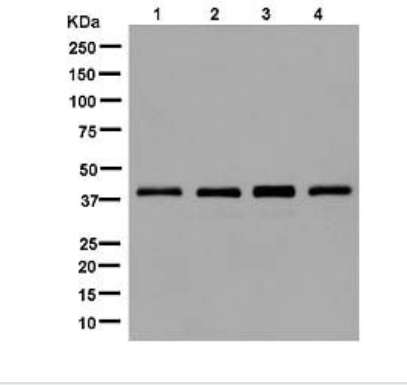
Lane 1: Wild-type HAP1 cell lysate (20 µg)
Lane 2: RING2 / RING1B / RNF2 knockout HAP1 cell lysate (20 µg)
Lane 3: HeLa cell lysate (20 µg)
Lane 4: Jurkat cell lysate (20 µg)
Lanes 1 - 4: Merged signal (red and green). Green - ab181140 observed at 42 kDa. Red - loading control, **ab7291**, observed at 52 kDa.

ab181140 was shown to recognize RING2 / RING1B / RNF2 when RING2 / RING1B / RNF2 knockout samples were used, along with additional cross-reactive bands. Wild-type and RING2 / RING1B / RNF2 knockout samples were subjected to SDS-PAGE. ab181140 at a dilution of 1/1000 and **ab7291** (loading control to alpha Tubulin) at a dilution of 1/10,000 were incubated overnight at 4°C. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preadsorbed (**ab216776**) secondary antibodies at 1/10,000 dilution for 1 h at room temperature before imaging.



Flow Cytometry (Intracellular) - Anti-RING2 / RING1B / RNF2 antibody [EPR12245] (ab181140)

Intracellular Flow Cytometry analysis of HepG2 (human hepatocellular carcinoma) cells labeling RING2 / RING1B / RNF2 with purified ab181140 at 1/70 dilution (10ug/ml) (red). Cells were fixed with 4% paraformaldehyde and permeabilised with 90% methanol. A Goat anti rabbit IgG (Alexa Fluor® 488) (1/2000 dilution) was used as the secondary antibody. Rabbit monoclonal IgG (Black) was used as the isotype control, cells without incubation with primary antibody and secondary antibody (Blue) were used as the unlabeled control.



Western blot - Anti-RING2 / RING1B / RNF2 antibody [EPR12245] (ab181140)

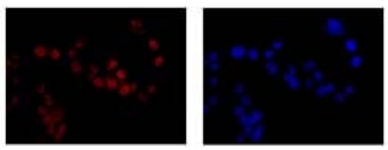
All lanes : Anti-RING2 / RING1B / RNF2 antibody [EPR12245] (ab181140) at 1/10000 dilution

Lane 1 : HeLa cell lysate
Lane 2 : 293 cell lysate
Lane 3 : Jurkat cell lysate
Lane 4 : HepG2 cell lysate

Lysates/proteins at 20 µg per lane.

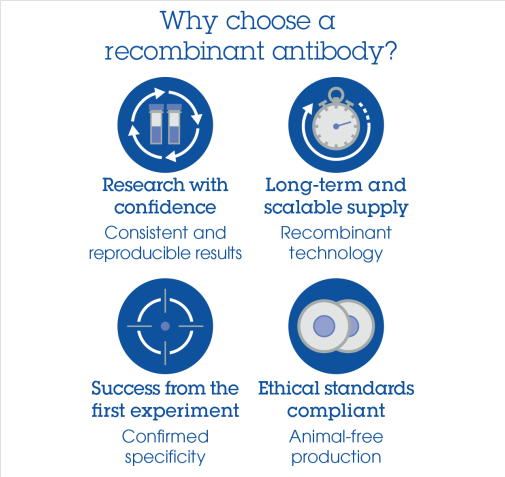
Secondary
All lanes : Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugate at 1/1000 dilution

Predicted band size: 38 kDa



Immunocytochemistry/ Immunofluorescence - Anti-RING2 / RING1B / RNF2 antibody [EPR12245] (ab181140)

Immunofluorescent analysis of HepG2 cells (paraformaldehyde-fixed, 4%) labeling RING2 / RING1B / RNF2 with ab181140 at 1/250 dilution followed by Goat anti rabbit IgG (Alexa Fluor® 555) secondary at 1/200 dilution and counter-stained with DAPI (blue).



Anti-RING2 / RING1B / RNF2 antibody [EPR12245]

(ab181140)

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