abcam

Product datasheet

Anti-ARID1A antibody [EPR13501] ab182560

KO VALIDATED Recombinant RabMAb

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Overview

Product name	Anti-ARID1A antibody [EPR13501]
Description	Rabbit monoclonal [EPR13501] to ARID1A
Host species	Rabbit
Tested applications	**Suitable for:** WB, ChIC/CUT&RUN-seq, Flow Cyt (Intra), IHC-P, ICC/IF **Unsuitable for:** ChIP
Species reactivity	**Reacts with:** Human **Predicted to work with:** Mouse, Rat
Immunogen	Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.
Positive control	IHC-P: Human kidney and human adenocarcinoma of endometrium without ARID1A mutation tissues. ICC/IF: Wildtype HAP1 and SH-SY5Y cells. WB: HEK-293T and SH-SY5Y cell lysates. ChIC/CUT&RUN-Seq: HCT116 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	pH: 7.2 Preservative: 0.01% Sodium azide Constituents: 40% Glycerol, 59% PBS, 0.05% BSA
Purity	Protein A purified
Clonality	Monoclonal

Clone number	EPR13501
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab182560 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. Predicted molecular weight: 242 kDa.
ChIC/CUT&RUN-seq		Use at an assay dependent concentration. 5µg
Flow Cyt (Intra)		Use at an assay dependent concentration. **ab172730** - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.
IHC-P	★★★★☆ (1)	1/1000. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.
ICC/IF		1/500.

Application notes Is unsuitable for ChIP.

Target

Function Involved in transcriptional activation and repression of select genes by chromatin remodeling (alteration of DNA-nucleosome topology). Binds DNA non-specifically. Also involved in vitamin D-coupled transcription regulation via its association with the WINAC complex, a chromatin-remodeling complex recruited by vitamin D receptor (VDR), which is required for the ligand-bound VDR-mediated transrepression of the CYP27B1 gene. Belongs to the neural progenitors-specific chromatin remodeling complex (npBAF complex) and the neuron-specific chromatin remodeling complex (nBAF complex). During neural development a switch from a stem/progenitor to a post-mitotic chromatin remodeling mechanism occurs as neurons exit the cell cycle and become committed to their adult state. The transition from proliferating neural stem/progenitor cells to post-mitotic neurons requires a switch in subunit composition of the npBAF and nBAF complexes. As neural progenitors exit mitosis and differentiate into neurons, npBAF complexes which contain ACTL6A/BAF53A and PHF10/BAF45A, are exchanged for homologous alternative ACTL6B/BAF53B and DPF1/BAF45B or DPF3/BAF45C subunits in neuron-specific complexes (nBAF). The npBAF complex is essential for the self-renewal/proliferative capacity of the multipotent neural stem cells. The nBAF complex along with CREST plays a role regulating the activity of genes essential for dendrite growth.

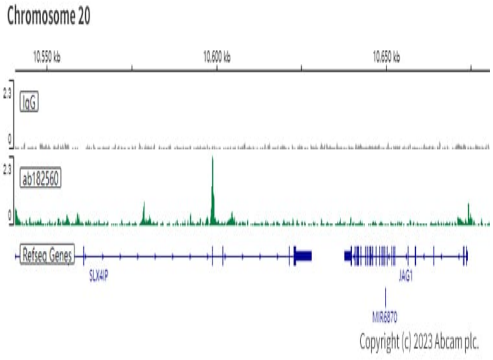
Tissue specificity Highly expressed in spleen, thymus, prostate, testis, ovary, small intestine, colon, and PBL, and at a much lower level in heart, brain, placenta, lung, liver, skeletal muscle, kidney, and pancreas.

Sequence similarities Contains 1 ARID domain.

Post-translational modifications Phosphorylated upon DNA damage, probably by ATM or ATR.

Cellular localization Nucleus.

Images

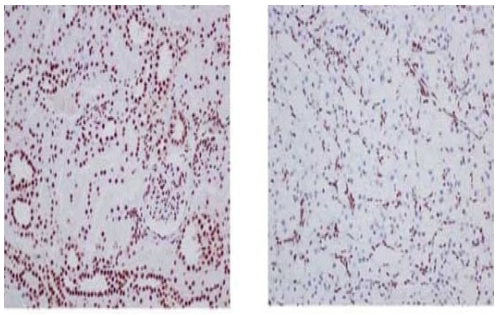


ChIC/CUT&RUN sequencing - Anti-ARID1A antibody [EPR13501] (ab182560)

ChIC/CUT&RUN was performed using a pAG-MNAse at a final concentration of 700 ng/mL, 2.5 x 10^5 HCT116 cells and 5µg of ab182560 [EPR13501]. The resulting DNA was sequenced on the Illumina NovaSeq 6000 to a depth of 10 million reads. The negative IgG control **ab172730** is also shown.

Additional screenshots of mapped reads can be downloaded **here**.

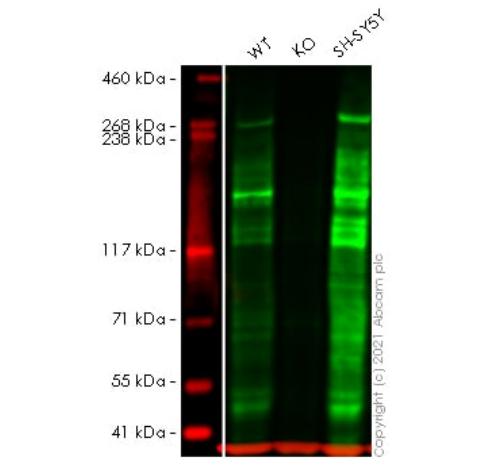
The University of Geneva owns patents relevant to ChIC (Chromatin Immuno-Cleavage) methods.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-ARID1A antibody [EPR13501] (ab182560)

Immunohistochemical analysis of paraffin embedded Human kidney tissue (Left image) labeling ARID1A using ab182560 at 1/1000 dilution. Right image: Right picture: paraffine embedded human clear cell carcinoma of kidney with ARID1A mutation. A Ready to use HRP Polymer for Rabbit IgG (prediluted) was used as secondary. Counterstain: Hematoxylin.

Heat mediated antigen retrieval with Tris-EDTA, pH 9 was performed before commencing with IHC staining protocol.



Western blot - Anti-ARID1A antibody [EPR13501] (ab182560)

All lanes : Anti-ARID1A antibody [EPR13501] (ab182560) at 1/1000 dilution

Lane 1 : Wild-type HEK-293T cell lysate
Lane 2 : ARID1A knockout HEK-293T cell lysate
Lane 3 : SH-SY5Y cell lysate

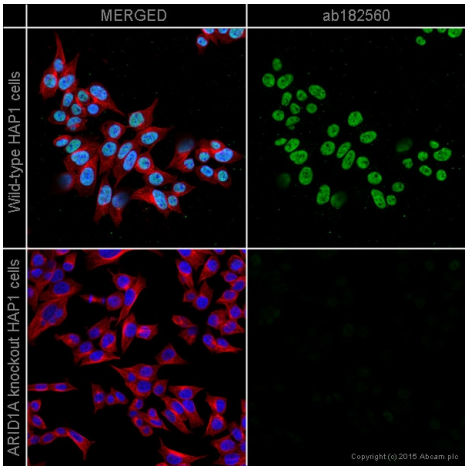
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 242 kDa
Observed band size: 270 kDa

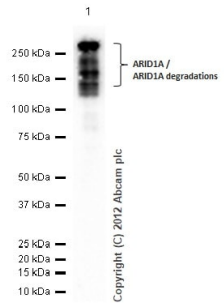
Lanes 1 - 3: Merged signal (red and green). Green - ab182560 observed at 270 kDa. Red - loading control **ab8245** (Mouse anti-GAPDH antibody [6C5]) observed at 37 kDa.

ab182560 was shown to react with ARID1A in wild-type HEK-293T cells in Western blot with loss of signal observed in ARID1A knockout cell line **ab266189** (ARID1A knockout cell lysate **ab257250**). Wild-type HEK-293T and ARID1A knockout cell lysates were subjected to SDS-PAGE. Membranes were blocked in 3 % milk in TBS-T (0.1 % Tween®) before incubation with ab182560 and **ab8245** (Mouse anti-GAPDH antibody [6C5]) overnight at 4°C at a 1 in 1000 dilution and a 1 in 20000 dilution respectively. Blots were incubated with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed (**ab216776**) secondary antibodies at 1 in 20000 dilution for 1 h at room temperature before imaging.



Immunocytochemistry/ Immunofluorescence - Anti-ARID1A antibody [EPR13501] (ab182560)

ab182560 staining ARID1A in wild-type HAP1 cells (top panel) and ARID1A knockout HAP1 cells (bottom panel). The cells were fixed with 4% formaldehyde (10min), permeabilized with 0.1% Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated with ab182560 at 1/500 dilution and **ab195889** at 1/250 dilution (shown in pseudo colour red) overnight at +4°C, followed by a further incubation at room temperature for 1h with a goat secondary antibody to Rabbit IgG (Alexa Fluor® 488) (**ab150081**) at 2 µg/ml (shown in green). Nuclear DNA was labelled in blue with DAPI.



Western blot - Anti-ARID1A antibody [EPR13501] (ab182560)

Anti-ARID1A antibody [EPR13501] (ab182560) at 1/1000 dilution + 293T (Human embryonic kidney epithelial cell) whole cell lysate at 20 µg

Secondary

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

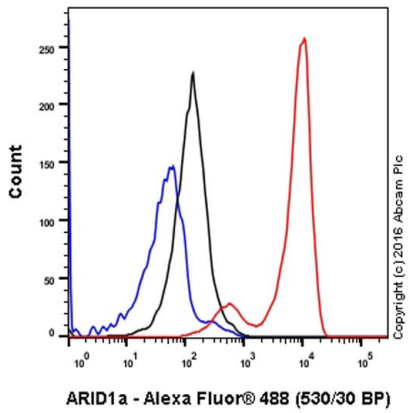
Predicted band size: 242 kDa
Observed band size: 130,270 kDa

Exposure time: 20 seconds

Blocking buffer and concentration: 5% NFDM/TBS

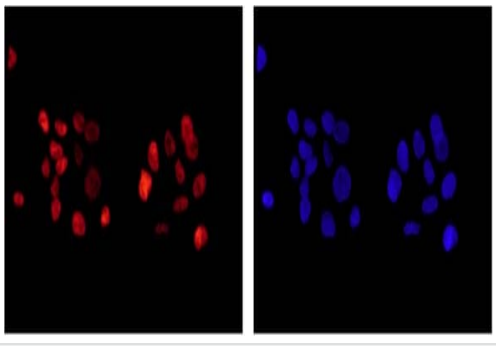
Observed band: 130-270 kDa

ARID1A has many mutations which typically generate truncated proteins that are highly prone to degradation. (PMID: 21614196, PMID: 29486633, PMID: 34429326).



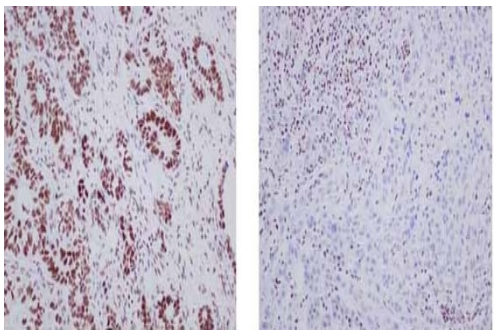
Flow Cytometry (Intracellular) - Anti-ARID1A antibody [EPR13501] (ab182560)

Intracellular Flow Cytometry analysis of SH-SY5Y (human neuroblastoma) cells labeling ARID1A with purified ab182560 at 1/230 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.



Immunocytochemistry/ Immunofluorescence - Anti-ARID1A antibody [EPR13501] (ab182560)

Immunofluorescent analysis of SH-SY5Y cells labeling ARID1A with ab182560 at 1/500 and Goat anti rabbit IgG(Alexa Fluor®555) at 1/200. Image at the right stained with DAPI.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-ARID1A antibody [EPR13501] (ab182560)

Immunohistochemical analysis of paraffin embedded Human adenocarcinoma of endometrium without ARID1A mutation (Left image) labeling ARID1A using ab182560 at 1/1000 dilution. Right image: Right picture: paraffine embedded human adenocarcinoma of endometrium with ARID1A mutation. A Ready to use HRP Polymer for Rabbit IgG (prediluted) was used as secondary. Counterstain: Hematoxylin.

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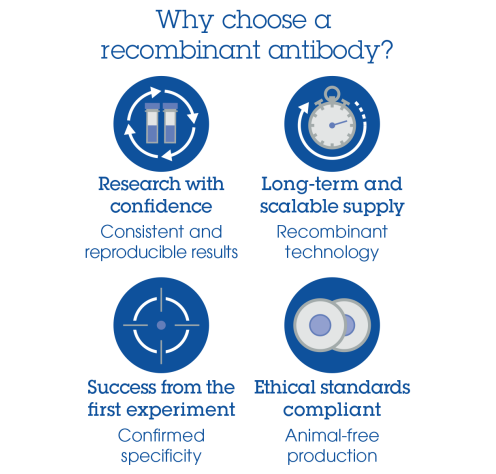

Anti-ARID1A antibody [EPR13501] (ab182560)

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