abcam

Product datasheet

Anti-BRG1 antibody [EPNCIR111A] - BSA and Azide free ab215998

KO VALIDATED Recombinant RabMAb

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Overview

Product name	Anti-BRG1 antibody [EPNCIR111A] - BSA and Azide free
Description	Rabbit monoclonal [EPNCIR111A] to BRG1 - BSA and Azide free
Host species	Rabbit
Tested applications	**Suitable for:** Flow Cyt (Intra), ChIC/CUT&RUN-seq, IHC-P, WB, IP, ICC/IF
Species reactivity	**Reacts with:** Mouse, Rat, Human
Immunogen	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
Positive control	WB: Hap1, HEK-293T, K562, HeLa, MOLT4, NIH3T3, RAW 264.7, C6 and PC12 cell lysates; Human kidney and testis tissue lysates. IHC-P: Human testis, colon, cervical carcinoma and kidney tissue, rat liver tissue and mouse kidney tissue. ICC/IF: HeLa cells, Raji and SMARCA4-HAP1 cells. Flow Cyt (intra): HeLa and HAP1 cells. ChIC/CUT&RUN-Seq: HeLa cells.
General notes	ab215998 is the carrier-free version of **ab110641**. Our **carrier-free** antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. The carrier-free buffer and high concentration allow for increased conjugation efficiency. This conjugation-ready format is designed for use with fluorochromes, metal isotopes, oligonucleotides, and enzymes, which makes them ideal for antibody labelling, functional and cell-based assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications. Use our **conjugation kits** for antibody conjugates that are ready-to-use in as little as 20 minutes with <1 minute hands-on-time and 100% antibody recovery: available for fluorescent dyes, HRP, biotin and gold. This product is compatible with the Maxpar® Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar® is a trademark of Fluidigm Canada Inc. 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**.

This antibody was developed as part of a collaboration between Epitomics, the National Cancer Institute's Center for Cancer Research and the lab of Gordon Hager. **View antibodies from NCI Center for Cancer Research Collaboration**.

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C. Do Not Freeze.
Storage buffer	pH: 7.20 Constituent: PBS
Carrier free	Yes
Purity	Protein A purified
Clonality	Monoclonal
Clone number	EPNCIR111A
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab215998 in the following tested applications.

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

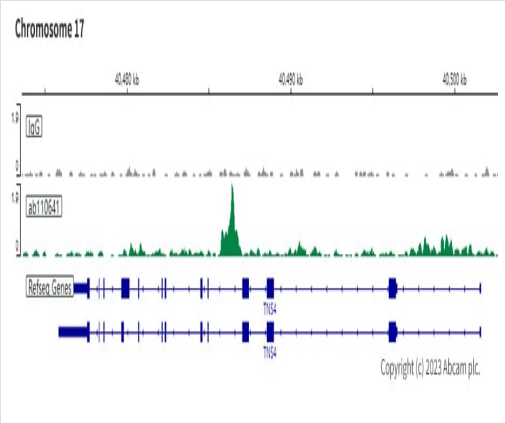
Application	Abreviews	Notes
Flow Cyt (Intra)		Use at an assay dependent concentration.
ChIC/CUT&RUN-seq		Use at an assay dependent concentration.
IHC-P		Use at an assay dependent concentration. Perform heat mediated antigen retrieval before commencing with IHC staining protocol. Heat up to 98°C, below boiling, and then let cool for 10-20 min.
WB		Use at an assay dependent concentration. Predicted molecular weight: 185 kDa.
IP		Use at an assay dependent concentration.
ICC/IF		Use at an assay dependent concentration.

Target

Function Transcriptional coactivator cooperating with nuclear hormone receptors to potentiate transcriptional activation. Component of the CREST-BRG1 complex, a multiprotein complex that

	regulates promoter activation by orchestrating a calcium-dependent release of a repressor complex and a recruitment of an activator complex. In resting neurons, transcription of the c-FOS promoter is inhibited by BRG1-dependent recruitment of a phospho-RB1-HDAC repressor complex. Upon calcium influx, RB1 is dephosphorylated by calcineurin, which leads to release of the repressor complex. At the same time, there is increased recruitment of CREBBP to the promoter by a CREST-dependent mechanism, which leads to transcriptional activation. The CREST-BRG1 complex also binds to the NR2B promoter, and activity-dependent induction of NR2B expression involves a release of HDAC1 and recruitment of CREBBP. 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. SMARCA4/BAF190A may promote neural stem cell self-renewal/proliferation by enhancing Notch-dependent proliferative signals, while concurrently making the neural stem cell insensitive to SHH-dependent differentiating cues (By similarity). 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. Acts as a corepressor of ZEB1 to regulate E-cadherin transcription and is required for induction of epithelial-mesenchymal transition (EMT) by ZEB1.
Tissue specificity	Colocalizes with ZEB1 in E-cadherin-negative cells from established lines, and stroma of normal colon as well as in de-differentiated epithelial cells at the invasion front of colorectal carcinomas (at protein level).
Involvement in disease	Defects in SMARCA4 are the cause of rhabdoid tumor predisposition syndrome type 2 (RTPS2) [MIM:613325]. RTPS2 is a familial cancer syndrome predisposing to renal or extrarenal malignant rhabdoid tumors and to a variety of tumors of the central nervous system, including choroid plexus carcinoma, medulloblastoma, and central primitive neuroectodermal tumors. Rhabdoid tumors are the most aggressive and lethal malignancies occurring in early childhood.
Sequence similarities	Belongs to the SNF2/RAD54 helicase family. Contains 1 bromo domain. Contains 1 helicase ATP-binding domain. Contains 1 helicase C-terminal domain. Contains 1 HSA domain.
Post-translational modifications	Phosphorylated upon DNA damage, probably by ATM or ATR.
Cellular localization	Nucleus.

Images



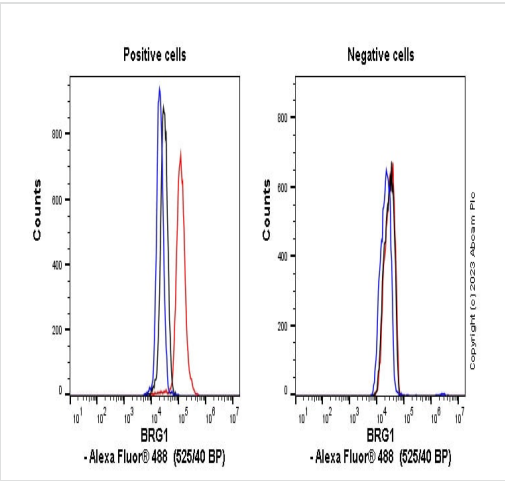
ChIC/CUT&RUN sequencing - Anti-BRG1 antibody [EPNCIR111A] - BSA and Azide free (ab215998)

ChIC/CUT&RUN was performed using a pAG-MNAse at a final concentration of 700 ng/mL, 2.5 x 10^5 HeLa (Human cervix adenocarcinoma epithelial cell line) cells and 5µg of **ab110641** [EPNCIR111A]. 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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab110641**).



Flow Cytometry (Intracellular) - Anti-BRG1 antibody [EPNCIR111A] - BSA and Azide free (ab215998)

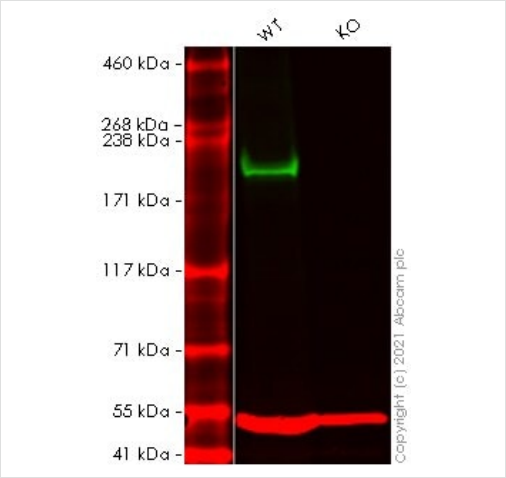
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab110641**).

Flow cytometry overlay histogram showing left wild-type Hap1 positive cells and right negative SMARCA4 knockout Hap1 stained with **ab95363** (red line). The cells were fixed with 80% methanol (5 min) and then permeabilised with 0.1% PBS-Triton X-100 for 15 min. The cells were then incubated in 1x PBS containing 10% normal goat serum to block non-specific protein-protein interaction followed by the antibody (**ab110641**) (1x 10^6 in 100µl at 0.008 µg/ml (1/266250)) for 30min at 22°C.

The secondary antibody Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed was incubated at 1/4000 for 30min at 22°C

Isotype control antibody Recombinant Rabbit IgG, monoclonal [EPR25A] - Isotype Control (black line) was used at the same concentration and conditions as the primary antibody. Unlabelled sample was also used as a control (blue line).

Acquisition of >5000 events were collected using a 50 mW Blue laser (488nm) and 525/40 bandpass filter.



Western blot - Anti-BRG1 antibody [EPNCIR111A] - BSA and Azide free (ab215998)

All lanes : Anti-BRG1 antibody [EPNCIR111A] (**ab110641**) at 1/1000 dilution

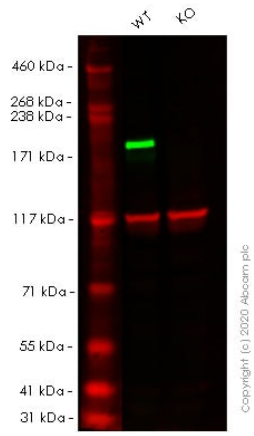
Lane 1 : Wild-type HEK-293T cell lysate
Lane 2 : SMARCA4 knockout HEK-293T cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 185 kDa
Observed band size: 185 kDa

False colour image of Western blot: Anti-BRG1 antibody [EPNCIR111A] staining at 1/1000 dilution, shown in green; Mouse anti-Alpha Tubulin [DM1A] (**ab7291**) loading control staining at 1/20000 dilution, shown in red. In Western blot, **ab110641** was shown to bind specifically to BRG1. A band was observed at 185 kDa in wild-type HEK-293T cell lysates with no signal observed at this size in SMARCA4 knockout cell line **ab255432** (knockout cell lysate **ab263853**). To generate this image, wild-type and SMARCA4 knockout HEK-293T cell lysates were analysed. First, samples were run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. Membranes were blocked in 3 % milk in TBS-0.1 % Tween® 20 (TBS-T) before incubation with primary antibodies overnight at 4 °C. Blots were washed four times in TBS-T, incubated with secondary antibodies for 1 h at room temperature, washed again four times then imaged. Secondary antibodies used were Goat anti-Rabbit IgG H&L (IRDye® 800CW) preabsorbed (**ab216773**) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed (**ab216776**) at 1/20000 dilution.



Western blot - Anti-BRG1 antibody [EPNCIR111A] - BSA and Azide free (ab215998)

All lanes : Anti-BRG1 antibody [EPNCIR111A] (**ab110641**) at 1/10000 dilution

Lane 1 : Wild-type HEK-293T cell lysate
Lane 2 : SMARCA4 knockout HEK-293T cell lysate

Lysates/proteins at 20 µg per lane.

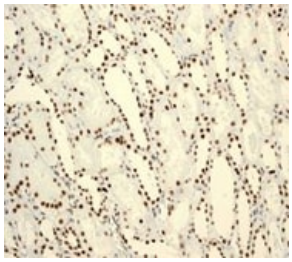
Performed under reducing conditions.

Predicted band size: 185 kDa
Observed band size: 185 kDa

This data was developed using the same antibody clone in a different buffer formulation (**ab110641**).

Lanes 1- 2: Merged signal (red and green). Green - **ab110641** observed at 185 kDa. Red - Anti-alpha Tubulin antibody [DM1A] - Loading Control (**ab7291**) observed at 50 kDa.

ab110641 was shown to react with SMARCA4 in wild-type HEK-293T cells in western blot. Loss of signal was observed when knockout cell line **ab255432** (knockout cell lysate **ab263853**) was used. Wild-type HEK-293T and SMARCA4 knockout HEK-293T 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. **ab110641** and Anti-alpha Tubulin antibody [DM1A] - Loading Control (**ab7291**) overnight at 4°C at a 1 in 10000 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.

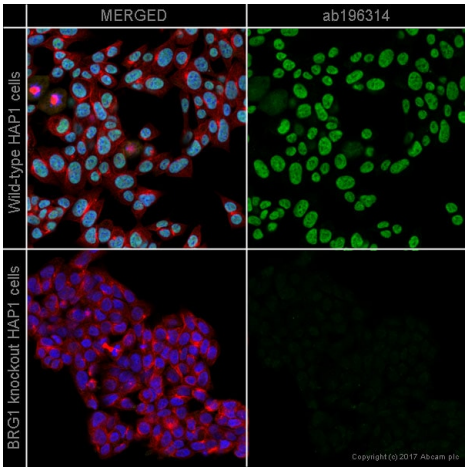


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-BRG1 antibody [EPNCIR111A] - BSA and Azide free (ab215998)

ab110641 at 1/100 dilution staining BRG1 in Human kidney tissue by Immunohistochemistry, Paraffin-embedded tissue.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab110641**).

Perform heat mediated antigen retrieval before commencing with IHC staining protocol.

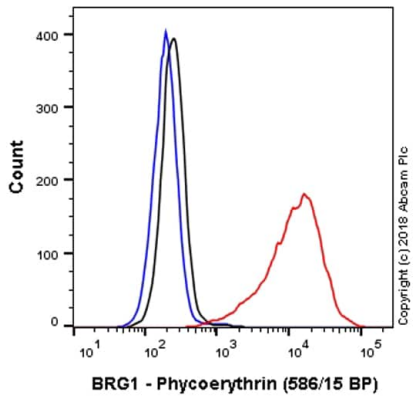


Immunocytochemistry/ Immunofluorescence - Anti-BRG1 antibody [EPNCIR111A] - BSA and Azide free (ab215998)

Clone EPNCIR111A (ab215998) has been successfully conjugated by Abcam. This image was generated using Anti-BRG1 antibody [EPNCIR111A] (Alexa Fluor® 488). Please refer to **ab196314** for protocol details.

ab196314 staining BRG1 in wild-type HAP1 cells (top panel) and BRG1 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 overnight at +4°C with **ab196314** at a 1/500 dilution (shown in green) and **ab195889**, Mouse monoclonal to alpha Tubulin (Alexa Fluor® 594), at a 1/250 dilution (shown in red). Nuclear DNA was labelled with DAPI (shown in blue).

Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).



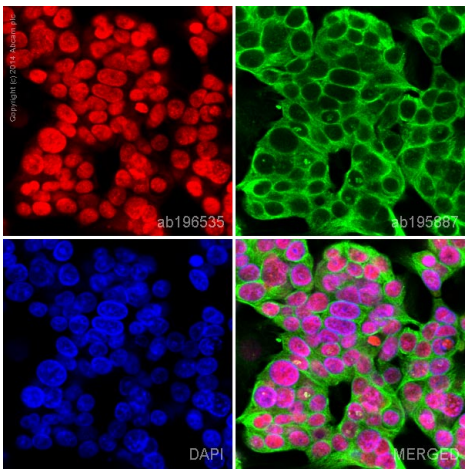
Flow Cytometry (Intracellular) - Anti-BRG1 antibody [EPNCIR111A] - BSA and Azide free (ab215998)

Clone EPNCIR111A (ab215998) has been successfully conjugated by Abcam. This image was generated using Anti-BRG1 antibody [EPNCIR111A] (PE). Please refer to **ab225124** for protocol details.

Overlay histogram showing HeLa cells stained with **ab225124** (red line). The cells were fixed with 4% formaldehyde (10 min) and then permeabilized with 0.1% PBS-Triton X-100 for 15 min. The cells were then incubated in 1x PBS / 10% normal goat serum to block non-specific protein-protein interactions followed by the antibody (**ab225124**, 1/500 dilution) for 30 min at 22°C.

Isotype control antibody (black line) was Rabbit IgG (monoclonal) Phycoerythrin (**ab209478**) used at the same concentration and conditions as the primary antibody. Unlabelled sample (blue line) was also used as a control.

Acquisition of >5,000 events were collected using a 50 mW Yellow/Green laser (561nm) and 586/15 bandpass filter.

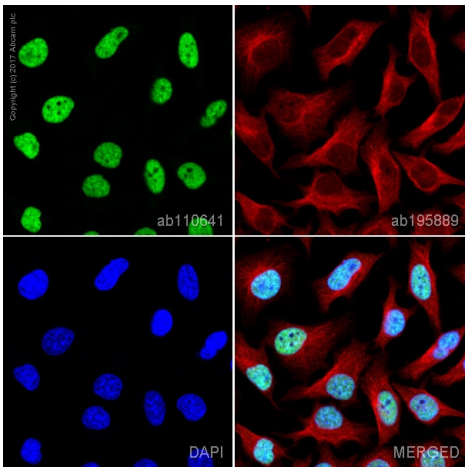


Immunocytochemistry/ Immunofluorescence - Anti-BRG1 antibody [EPNCIR111A] - BSA and Azide free (ab215998)

Clone EPNCIR111A (ab215998) has been successfully conjugated by Abcam. This image was generated using Anti-BRG1 antibody [EPNCIR111A] (Alexa Fluor® 647). Please refer to **ab196535** for protocol details.

ab196535 staining BRG1 in SW480 cells. The cells were fixed with 100% methanol (5 min), 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 overnight at +4°C with **ab196535** at a 1/100 dilution (shown in red) and **ab195887**, Mouse monoclonal to alpha Tubulin (Alexa Fluor® 488), at a 1/250 dilution (shown in green). Nuclear DNA was labelled with DAPI (shown in blue).

Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).



Immunocytochemistry/ Immunofluorescence - Anti-BRG1 antibody [EPNCIR111A] - BSA and Azide free (ab215998)

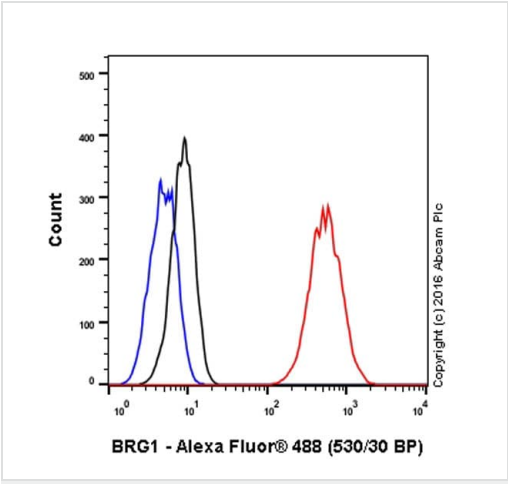
ab110641 staining BRG1 in HeLa cells. The cells were fixed with 4% formaldehyde (10min), permeabilized with 0.1% Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated with **ab110641** at 1/500 dilution and **ab195889** (Mouse monoclonal [DM1A] to alpha Tubulin - Microtubule Marker (Alexa Fluor® 594)) at 1/250 dilution overnight at +4°C, followed by a further incubation at room temperature for 1h with **ab150081** (Goat polyclonal Secondary Antibody to Rabbit IgG - H&L (Alexa Fluor® 488)) at 2 µg/ml (shown in green). Nuclear DNA was labelled in blue with DAPI.

Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab110641**).

Western blot - Anti-BRG1 antibody [EPNCIR111A] - BSA and Azide free (ab215998)

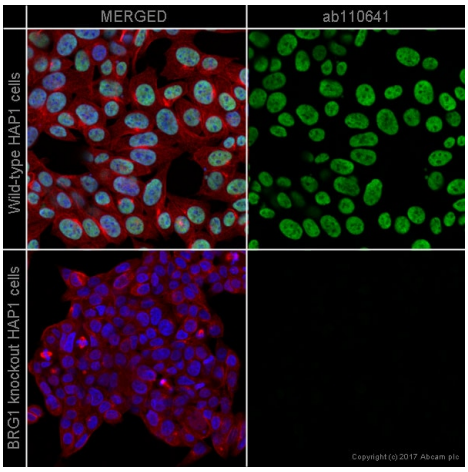
This data was developed using **ab110641**, the same antibody clone in a different buffer formulation. Different batches of **ab110641** were tested on HeLa (Human cervix adenocarcinoma epithelial cell) lysate at 0.5 µg/ml. 15 µg of lysate was loaded in each lane. Bands observed at 185 kDa.



Flow Cytometry (Intracellular) - Anti-BRG1 antibody [EPNCIR111A] - BSA and Azide free (ab215998)

Intracellular Flow Cytometry analysis of HeLa (human cervix adenocarcinoma) cells labeling BRG1 with purified **ab110641** at 1/200 dilution (10µg/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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab110641**).

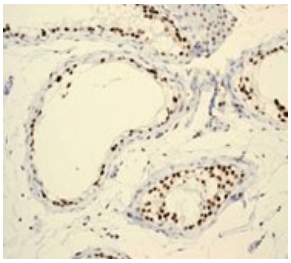


Immunocytochemistry/ Immunofluorescence - Anti-BRG1 antibody [EPNCIR111A] - BSA and Azide free (ab215998)

This ICC/IF data was generated using the same anti-BRG1 antibody clone, EPNCIR111A, in a different buffer formulation (cat# **ab110641**).

ab110641 staining BRG1 in wild-type HAP1 cells (top panel) and BRG1 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 **ab110641** at 1/500 dilution and **ab195889** at 1/250 dilution (shown in pseudocolour 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.

Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-BRG1 antibody [EPNCIR111A] - BSA and Azide free (ab215998)

This IHC data was generated using the same anti-BRG1 antibody clone, EPNCIR111A, in a different buffer formulation (cat# **ab110641**).

ab110641 at 1/100 dilution staining BRG1 in Human testis tissue by Immunohistochemistry, Paraffin-embedded tissue.

Perform heat mediated antigen retrieval before commencing with IHC staining protocol.

Anti-BRG1 antibody [EPNCIR111A] - BSA and Azide free (ab215998)

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