abcam

Product datasheet

Anti-BRG1 antibody [EPR3912] ab108318

敲除验证 重组 RabMAb

★★★★★ 2 Abreviews 7 References 22 图像

概述

产品名称	Anti-BRG1抗体[EPR3912]
描述	兔单克隆抗体[EPR3912] to BRG1
宿主	Rabbit
经测试应用	**适用于:** ChIC/CUT&RUN-seq, ICC/IF, WB, IHC-P, Flow Cyt (Intra), IP
种属反应性	**与反应:** Mouse, Rat, Human
免疫原	Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.
阳性对照	WB: HAP1, HEK-293T, K562, RAW264.7, NIH/3T3, PC-12 and Molt-4 cell lysate. ICC/IF: HeLa cells; SMARCA4-HAP1 cells. IHC-P: Human colon, urinary bladder transitional carcinoma, breast carcinoma, bladder cancer, ovarian carcinoma and normal tonsil tissue, mouse and rat stomach tissue. Flow Cyt (intra): HeLa cells IP: K562 cell lysate, NIH/3T3 cell lysate. ChIC/CUT&RUN-Seq: HeLa cells.
常规说明	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**.

性能

形式	Liquid
存放说明	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.
存储溶液	pH: 7.20 Preservative: 0.01% Sodium azide Constituents: 59% PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
纯度	Protein A purified
克隆	单克隆

克隆编号	EPR3912
同种型	IgG

应用

The Abpromise guarantee **Abpromise™承诺保证使用ab108318于以下的经测试应用**

"应用说明"部分 下显示的仅为推荐的起始稀释度;实际最佳的稀释度/浓度应由使用者检定。

应用	Ab评论	说明
ChIC/CUT&RUN-seq		Use at an assay dependent concentration. 5µg
ICC/IF		1/50. **For unpurified format use at 1/500 dilution.**
WB	★★★★★ (2)	1/1000 - 1/5000. Predicted molecular weight: 185 kDa.
IHC-P		1/100 - 1/500. Perform heat mediated antigen retrieval before commencing with IHC staining protocol. See **IHC antigen retrieval protocols**. Heat up to 98 degrees C, below boiling, and then let cool for 10-20 min.
Flow Cyt (Intra)		1/20.
IP		Use at an assay dependent concentration.

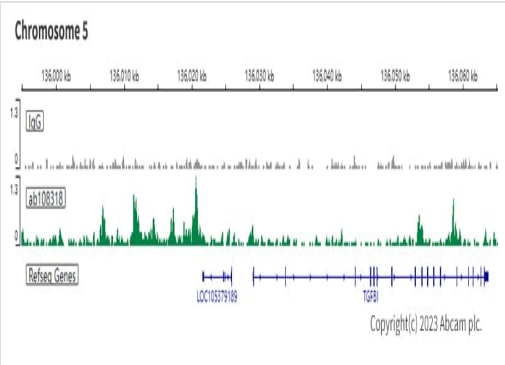
靶标

功能

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.
组织特异性	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).
疾病相关	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.
序列相似性	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.
翻译后修饰	Phosphorylated upon DNA damage, probably by ATM or ATR.
细胞定位	Nucleus.

图片

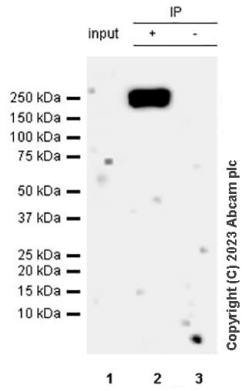


ChIC/CUT&RUN sequencing - Anti-BRG1 antibody [EPR3912] (ab108318)

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 ab108318 [EPR3912]. 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.



Immunoprecipitation - Anti-BRG1 antibody [EPR3912] (ab108318)

BRG1 was immunoprecipitated from NIH/3T3 (mouse embryonic fibroblast), whole cell lysate with ab108318 at 1/30 dilution (2µg in 0.35mg lysates). Western blot was performed on the immunoprecipitate using ab108318 at 1/1000 dilution. VeriBlot for IP secondary antibody(HRP)(**ab131366**) was used as secondary antibody at 1/5000 dilution.

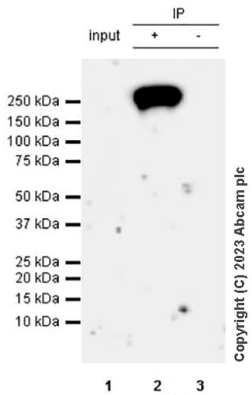
Lane 1 (Input): NIH/3T3 (mouse embryonic fibroblast), whole cell lysate, 10 µg

Lane 2 (+) : NIH/3T3 whole cell lysate

Lane 3 (-) : Rabbit monoclonal IgG (**ab172730**) instead of ab108318 in NIH/3T3 whole cell lysate

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

Observed MW: 185 kDa.



Immunoprecipitation - Anti-BRG1 antibody [EPR3912] (ab108318)

BRG1 was immunoprecipitated from K-562 (human chronic myelogenous leukemia lymphoblast), whole cell lysate with ab108318 at 1/30 dilution (2µg in 0.35mg lysates). Western blot was performed on the immunoprecipitate using ab108318 at 1/1000 dilution. VeriBlot for IP secondary antibody(HRP) (**ab131366**) was used as secondary antibody at 1/5000 dilution.

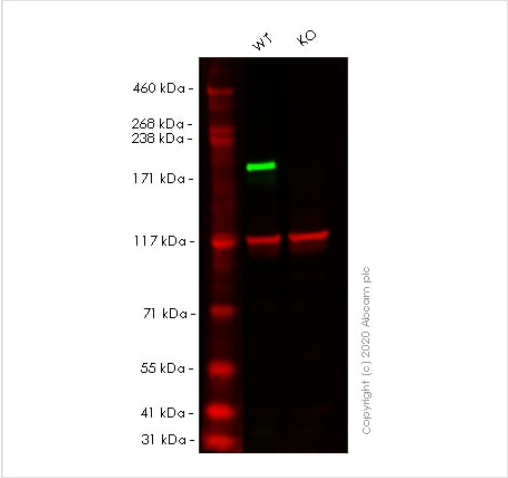
Lane 1 (Input): K-562 (human chronic myelogenous leukemia lymphoblast), whole cell lysate, 10 µg

Lane 2 (+) : K-562 whole cell lysate

Lane 3 (-) : Rabbit monoclonal IgG (**ab172730**) instead of ab108318 in K-562 whole cell lysate

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

Observed MW: 185 kDa.



Western blot - Anti-BRG1 antibody [EPR3912] (ab108318)

All lanes : Anti-BRG1 antibody [EPR3912] (ab108318) at 1/10000 dilution

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

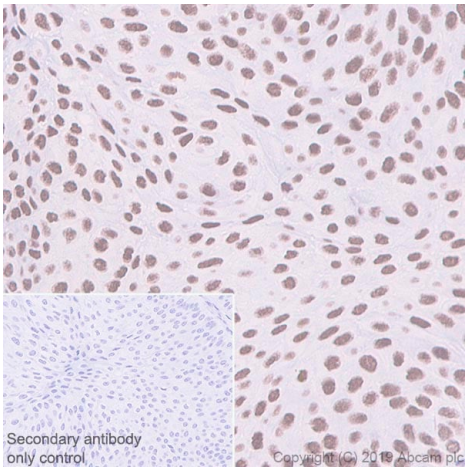
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

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

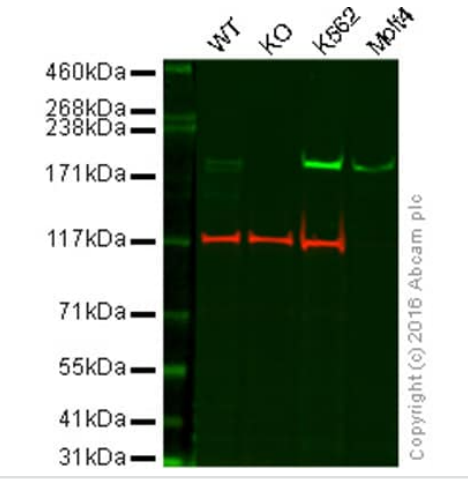
Lanes 1- 2: Merged signal (red and green). Green - ab108318 observed at 185 kDa. Red - Anti-Vinculin antibody [VIN-54] observed at 124 kDa.

ab108318 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. ab108318 and Anti-Vinculin antibody [VIN-54] 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 [EPR3912] (ab108318)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of Human bladder cancer tissue sections labeling BRG1 with purified ab108318 at 1/500 dilution (0.23 µg/ml). Heat mediated antigen retrieval was performed using **ab93684** (Tris/EDTA buffer, pH 9.0). ImmunoHistoProbe one step HRP Polymer (ready to use) was used as the secondary antibody. Negative control: PBS instead of the primary antibody. Hematoxylin was used as a counterstain.



Western blot - Anti-BRG1 antibody [EPR3912] (ab108318)

All lanes : Anti-BRG1 antibody [EPR3912] (ab108318) at 1/1000 dilution

Lane 1 : Wild-type HAP1 cell lysate
Lane 2 : BRG1 knockout HAP1 cell lysate
Lane 3 : K562 cell lysate
Lane 4 : Molt-4 cell lysate

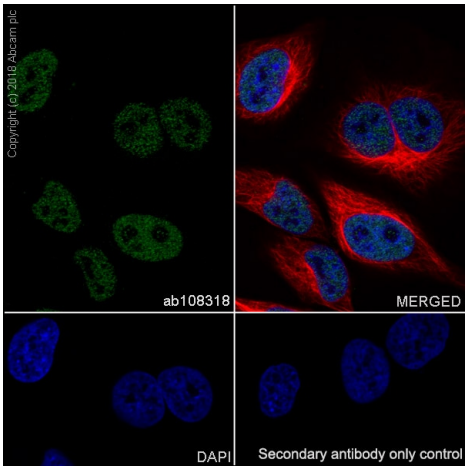
Lysates/proteins at 20 µg per lane.

Predicted band size: 185 kDa
Additional bands at: 185 kDa. We are unsure as to the identity of these extra bands.

Lanes 1 - 4: Merged signal (red and green). Red - loading control, **ab18058** (unpurified), observed at 124 kDa.

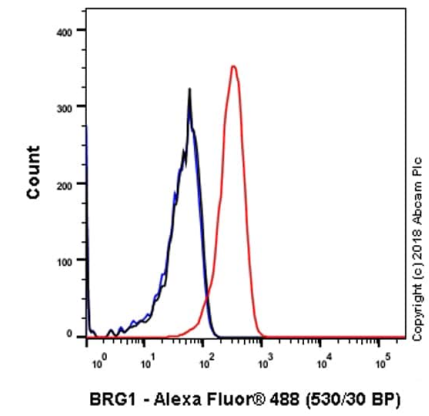
ab108318 was shown to specifically react with BRG1 in wild-type HAP1 cells along with additional cross reactive bands. No band was observed when BRG1 knockout samples were used. Wild-type and BRG1 knockout samples were subjected to SDS-PAGE,

ab108318 and **ab18058** (loading control to Vinculin) were diluted 1/1000 and 1/10,000 respectively and 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 1hr at room temperature before imaging.



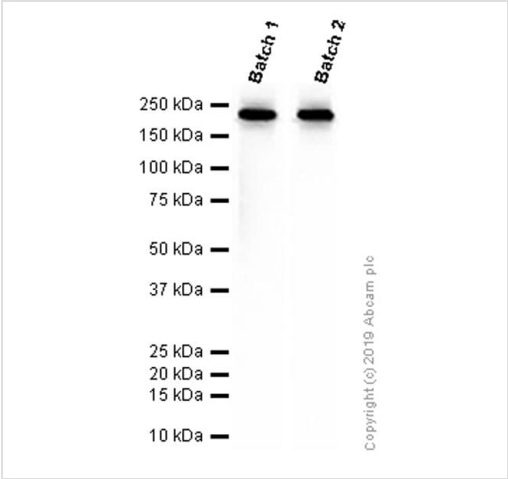
Immunocytochemistry/ Immunofluorescence analysis of HeLa (Human cervix adenocarcinoma epithelial cell) cells labeling BRG1 with purified ab108318 at 1/50 dilution (2.3 µg/ml). Cells were fixed in 4% Paraformaldehyde and permeabilized with 0.1% tritonX-100. Cells were counterstained with **ab195889** Anti-alpha Tubulin antibody [DM1A] - Microtubule Marker (Alexa Fluor® 594) at 1/200 (2.5 µg/ml). Goat anti rabbit IgG (Alexa Fluor® 488, **ab150077**) was used as the secondary antibody at 1/1000 (2 µg/ml) dilution. DAPI (blue) was used as nuclear counterstain. PBS instead of the primary antibody was used as the secondary antibody only control.

Immunocytochemistry/ Immunofluorescence - Anti-BRG1 antibody [EPR3912] (ab108318)



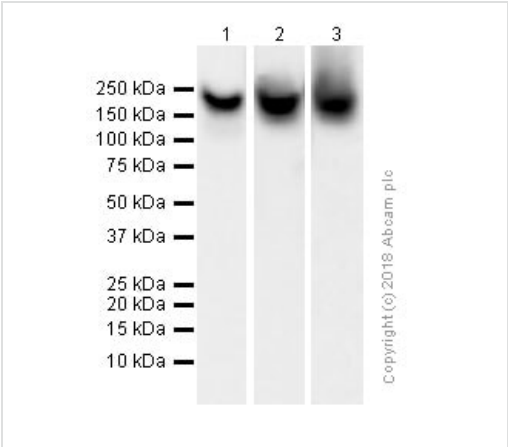
Intracellular Flow Cytometry analysis of HeLa (Human cervix adenocarcinoma epithelial cell) cells labeling BRG1 with purified ab108318 at 1/20 dilution (10µg/ml) (red). Cells were fixed with 4% Paraformaldehyde and permeabilised with 90% Methanol. A Goat anti rabbit IgG (Alexa Fluorr® 488, **ab150077**) secondary antibody was used at 1/2000. Isotype control - Rabbit monoclonal IgG (Black). Unlabeled control - Cell without incubation with primary antibody and secondary antibody (Blue).

Flow Cytometry (Intracellular) - Anti-BRG1 antibody [EPR3912] (ab108318)



Western blot - Anti-BRG1 antibody [EPR3912] (ab108318)

Different batches of ab108318 were tested on K-562 (Human chronic myelogenous leukemia lymphoblast) lysate at 0.05 µg/ml. 15 µg of lysate was loaded in each lane. Bands observed at 185 kDa.



Western blot - Anti-BRG1 antibody [EPR3912] (ab108318)

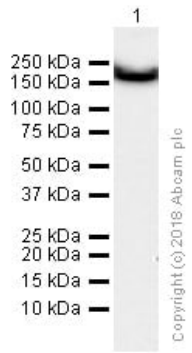
All lanes : Anti-BRG1 antibody [EPR3912] (ab108318) at 1/1000 dilution (Purified)

Lane 1 : K-562 (Human chronic myelogenous leukemia lymphoblast) whole cell lysates
Lane 2 : NIH/3T3 (Mouse embryonic fibroblast) whole cell lysates
Lane 3 : PC-12 (Rat adrenal gland pheochromocytoma) whole cell lysates

Lysates/proteins at 20 µg per lane.

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

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



Western blot - Anti-BRG1 antibody [EPR3912] (ab108318)

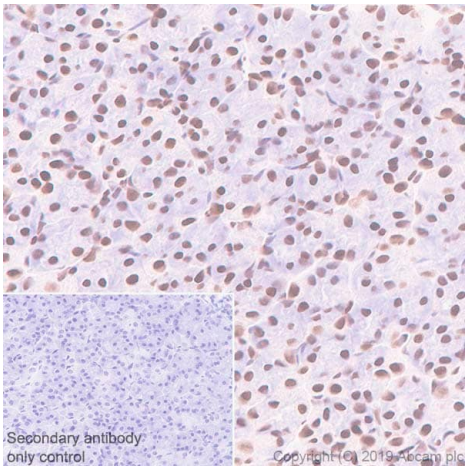
Anti-BRG1 antibody [EPR3912] (ab108318) at 1/10000 dilution (Purified) + RAW264.7 (Mouse Abelson murine leukemia virus-induced tumor macrophage) whole cell lysates at 20 µg

Secondary

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

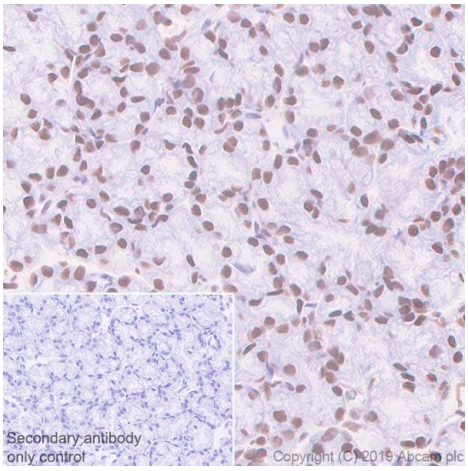
Predicted band size: 185 kDa

Observed band size: 185 kDa



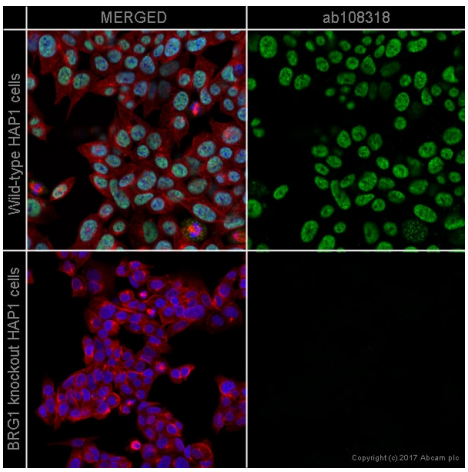
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-BRG1 antibody [EPR3912] (ab108318)

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of Rat stomach tissue sections labeling BRG1 with purified ab108318 at 1/500 dilution (0.23 µg/ml). Heat mediated antigen retrieval was performed using **ab93684** (Tris/EDTA buffer, pH 9.0). ImmunoHistoProbe one step HRP Polymer (ready to use) was used as the secondary antibody. Negative control: PBS instead of the primary antibody. Hematoxylin was used as a counterstain.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-BRG1 antibody [EPR3912] (ab108318)

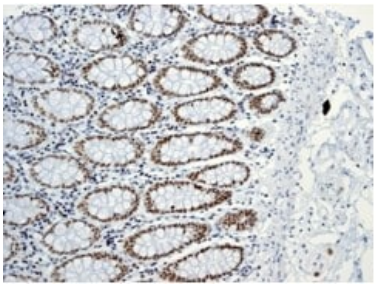
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) analysis of Mouse stomach tissue sections labeling BRG1 with purified ab108318 at 1/500 dilution (0.23 µg/ml). Heat mediated antigen retrieval was performed using **ab93684** (Tris/EDTA buffer, pH 9.0). ImmunoHistoProbe one step HRP Polymer (ready to use) was used as the secondary antibody. Negative control: PBS instead of the primary antibody. Hematoxylin was used as a counterstain.



Immunocytochemistry/ Immunofluorescence - Anti-BRG1 antibody [EPR3912] (ab108318)

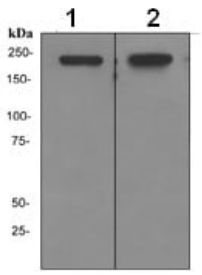
ab108318 (unpurified) staining BRG1 in wild-type HAP1 cells (top panel) and BRG1 knockout HAP1 cells (bottom panel). The cells were fixed with 4% formaldehyde (10min), permeabilized with 0.1% Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated with ab108318 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 [EPR3912] (ab108318)

Formalin-fixed paraffin-embedded human colon tissue stained for BRG1 using ab108318 at 1/100 dilution in immunohistochemical analysis. Heat mediated antigen retrieval was performed before commencing with IHC staining protocol.



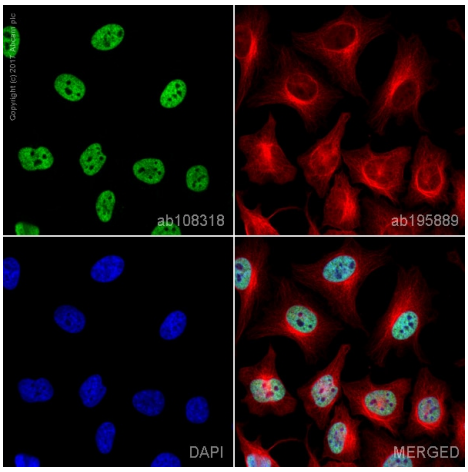
Western blot - Anti-BRG1 antibody [EPR3912] (ab108318)

All lanes : Anti-BRG1 antibody [EPR3912] (ab108318) at 1/1000 dilution (unpurified)

Lane 1 : K562 cell lysate
Lane 2 : Molt-4 cell lysate

Lysates/proteins at 10 µg per lane.

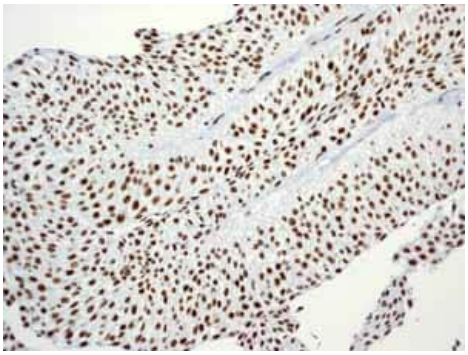
Predicted band size: 185 kDa



Immunocytochemistry/ Immunofluorescence - Anti-BRG1 antibody [EPR3912] (ab108318)

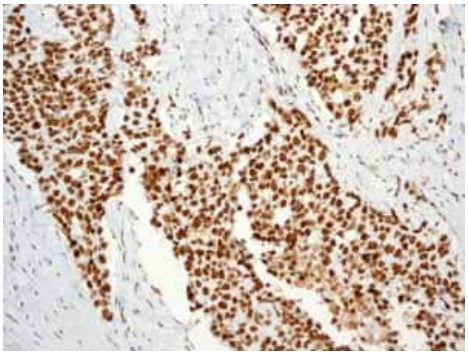
ab108318 (unpurified) staining BRG1 in HeLa cells. The cells were fixed with 4% formaldehyde (10min), permeabilized with 0.1% Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated with ab108318 at 1/500 dilution 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).



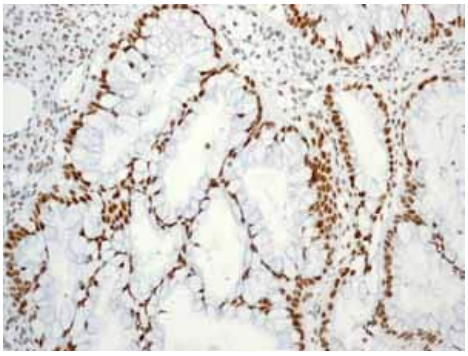
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-BRG1 antibody [EPR3912] (ab108318)

Formalin-fixed paraffin-embedded human Urinary bladder transitional carcinoma tissue.stained for BRG1 using ab108318 at 1/100 dilution in immunohistochemical analysis. Heat mediated antigen retrieval was performed before commencing with IHC staining protocol.



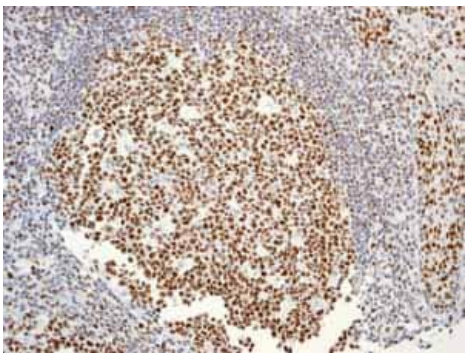
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-BRG1 antibody [EPR3912] (ab108318)

Formalin-fixed paraffin-embedded human Urinary Breast carcinoma tissue stained for BRG1 using ab108318 at 1/100 dilution in immunohistochemical analysis. Heat mediated antigen retrieval was performed before commencing with IHC staining protocol.



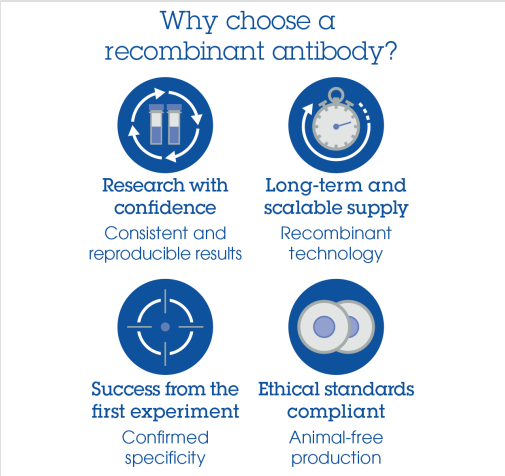
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-BRG1 antibody [EPR3912] (ab108318)

Formalin-fixed paraffin-embedded human Ovarian carcinoma tissue stained for BRG1 using ab108318 at 1/100 dilution in immunohistochemical analysis. Heat mediated antigen retrieval was performed before commencing with IHC staining protocol.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-BRG1 antibody [EPR3912] (ab108318)

Formalin-fixed paraffin-embedded Normal human tonsil tissue stained for BRG1 using ab108318 at 1/100 dilution in immunohistochemical analysis. Heat mediated antigen retrieval was performed before commencing with IHC staining protocol.



Anti-BRG1 antibody [EPR3912] (ab108318)

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