abcam

Product datasheet

Anti-BRD2 antibody [EPR7642] - ChIP Grade ab139690

敲除验证 重组 RabMAb

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概述

产品名称	Anti-BRD2抗体[EPR7642] - ChIP Grade
描述	兔单克隆抗体[EPR7642] to BRD2 - ChIP Grade
宿主	Rabbit
经测试应用	**适用于:** WB, ICC/IF, IHC-P, Flow Cyt (Intra), ChIP **不适用于:** IP
种属反应性	**与反应:** Human
免疫原	Synthetic peptide within Human BRD2 aa 1-100 (N terminal). The exact sequence is proprietary. Database link: **P25440**
阳性对照	WB: HEK293T, Jurkat, MOLT4, NCCIT and HeLa whole cell lysate (**ab150035**). ICC/IF: HeLa and wild-type HAP1 cells. IHC-P: Human testis tissue. ChIP: Nuclear extract from LNCaP 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 -20ºC.
存储溶液	pH: 7.2 Preservative: 0.01% Sodium azide Constituents: 9% PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA, 50% Tissue culture supernatant
纯度	Protein A purified
克隆	单克隆
克隆编号	EPR7642

同种型 IgG

应用

The Abpromise guarantee Abpromise™承诺保证使用ab139690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
WB	★★★★★ (2)	1/1000 - 1/10000. Predicted molecular weight: 88 kDa.
ICC/IF		Use a concentration of 0.5 µg/ml.
IHC-P	★★★★★ (2)	1/250 - 1/500. Perform heat mediated antigen retrieval before commencing with IHC staining protocol.
Flow Cyt (Intra)		1/100 - 1/1000. **ab172730** - Rabbit monoclonal IgG, is suitable for use as an isotype control with this antibody.
ChIP		Use at an assay dependent concentration.

应用说明 Is unsuitable for IP.

靶标

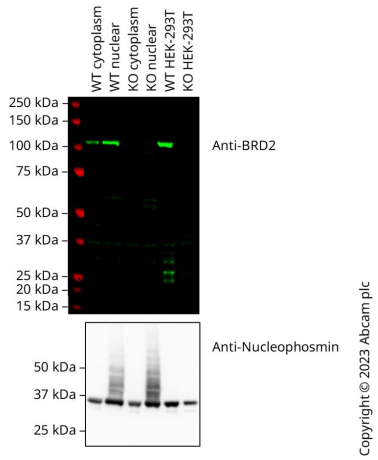
功能 May play a role in spermatogenesis or folliculogenesis.

序列相似性 Contains 2 bromo domains.
Contains 1 ET domain.

结构域 One bromodomain is sufficient for a partial interaction with histone H4 acetylated at 'Lys-13'.

细胞定位 Nucleus.

图片



Western blot - Anti-BRD2 antibody [EPR7642] - ChIP Grade (ab139690)

All lanes : Anti-BRD2 antibody [EPR7642] - ChIP Grade (ab139690) at 1/1000 dilution

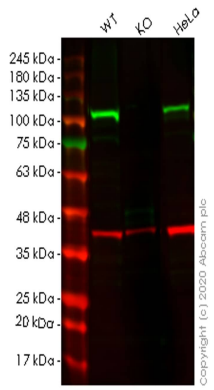
Lane 1 : Wild-type A549 **ab288558** cytoplasm cell lysate
Lane 2 : Wild-type A549 **ab288558** nuclear cell lysate
Lane 3 : BRD2 knockout A549 C7 cytoplasm cell lysate
Lane 4 : BRD2 knockout A549 C7 nuclear cell lysate
Lane 5 : Wild-type HEK-293T **ab255553** cell lysate
Lane 6 : BRD2 knockout HEK-293T cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 88 kDa
Observed band size: 110 kDa

False colour image of Western blot: Anti-BRD2 antibody [EPR7642] - ChIP Grade staining at 1/1000 dilution, shown in green; Mouse Anti-Nucleophosmin antibody [FC82291] (**ab10530**) loading control staining at 2 ug/mL imaged in ECL. In Western blot, ab139690 was shown to bind specifically to BRD2. A band was observed at 110 kDa in wild-type A549 cell lysates with no signal observed at this size in BRD2 knockout cell line. To generate this image, wild-type and BRD2 knockout A549 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 before development with Optiblot (ECL reagent **ab133456**) and imaged with 20 seconds exposure time. Secondary antibodies used were Goat anti-Rabbit IgG H&L 800CW and HRP conjugated Goat anti-Mouse (H+L) at 1/20000 dilution.



Western blot - Anti-BRD2 antibody [EPR7642] - ChIP Grade (ab139690)

All lanes : Anti-BRD2 antibody [EPR7642] - ChIP Grade (ab139690) at 1/1000 dilution

Lane 1 : Wild-type HEK293T cell lysate
Lane 2 : BRD2 knockout HEK293T cell lysate
Lane 3 : HeLa cell lysate

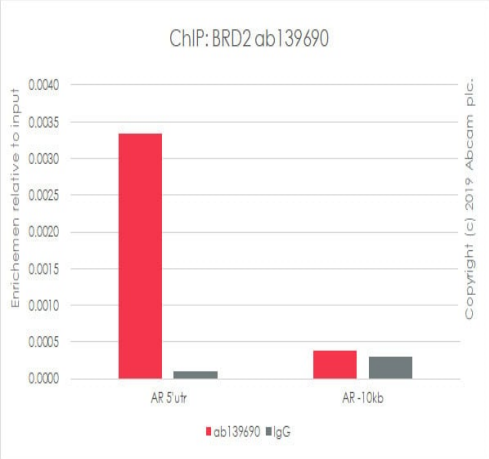
Lysates/proteins at 20 µg per lane.

Secondary
All lanes : Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (**ab216773**) at 1/10000 dilution

Predicted band size: 88 kDa
Observed band size: 110 kDa

Lanes 1-3: Merged signal (red and green). Green - ab139690 observed at 110 kDa. Red - loading control **ab8245** observed at 36 kDa.

ab139690 Anti-BRD2 antibody [EPR7642] was shown to specifically react with BRD2 in wild-type HEK293T cells. Loss of signal was observed when knockout cell line **ab267265** (knockout cell lysate **ab257191**) was used. Wild-type and BRD2 knockout samples were subjected to SDS-PAGE. ab139690 and Anti-GAPDH antibody [6C5] - Loading Control (**ab8245**) were incubated at room temperature for 2. 5 hours at 1 in 1000 dilution and 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.



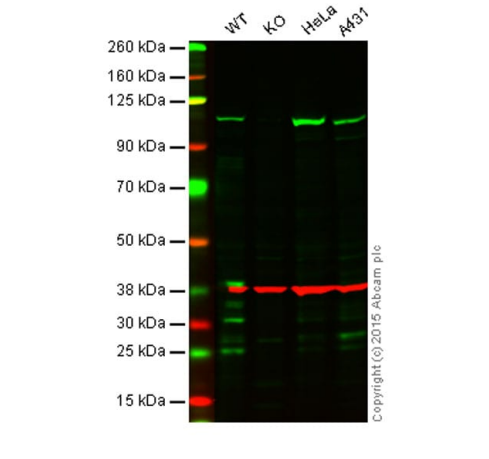
ChIP - Anti-BRD2 antibody [EPR7642] - ChIP Grade (ab139690)

Chromatin was prepared from LNCaP cells according to the Abcam Dual X-ChIP protocol*. Cells were fixed with formaldehyde for 10 minutes.

The ChIP was performed with 25 µg of chromatin, 5 µg of ab139690 (red), and 20 µl of Protein A/G sepharose beads. 5 µg of rabbit normal IgG was added to the beads control (gray). The immunoprecipitated DNA was quantified by real time PCR (Sybr green approach).

Primers and probes are located in the first kb of the transcribed region.

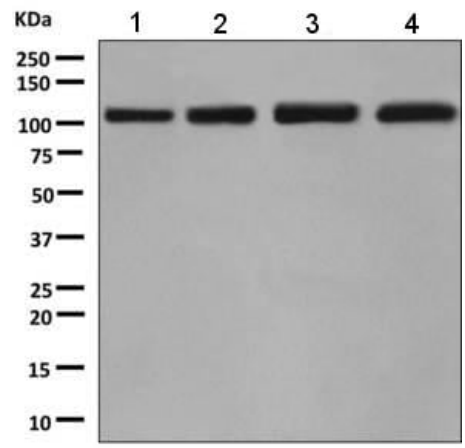
*http://www.abcam.com/resources?keywords=X%20ChIP%20protocol



Western blot - Anti-BRD2 antibody [EPR7642] - ChIP Grade (ab139690)

Lane 1: Wild-type HAP1 cell lysate (20 µg)
Lane 2: BRD2 knockout HAP1 cell lysate (20 µg)
Lane 3: HeLa cell lysate (20 µg)
Lane 4: A431 cell lysate (20 µg)
Lanes 1 - 4: Merged signal (red and green). Green - ab139690observed at 110 kDa. Red - loading control, **ab8245**, observed at 37 kDa.

ab139690 was shown to specifically react with BRD2 when BRD2 knockout samples were used. Wild-type and BRD2 knockout samples were subjected to SDS-PAGE. ab139690 and **ab8245** (loading control to GAPDH) were diluted 1/1000 and 1/2000 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 1 h at room temperature before imaging.



Western blot - Anti-BRD2 antibody [EPR7642] - ChIP Grade (ab139690)

All lanes : Anti-BRD2 antibody [EPR7642] - ChIP Grade (ab139690) at 1/1000 dilution

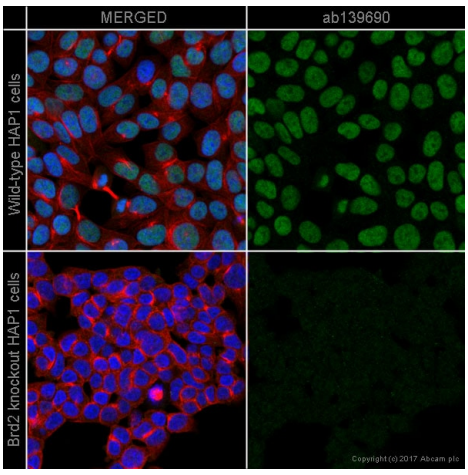
Lane 1 : MOLT4 cell lysate
Lane 2 : Jurkat cell lysate
Lane 3 : NCCIT cell lysate
Lane 4 : HeLa cell lysate

Lysates/proteins at 10 µg per lane.

Secondary
All lanes : Standard HRP labelled goat anti-rabbit at 1/2000 dilution

Developed using the ECL technique.

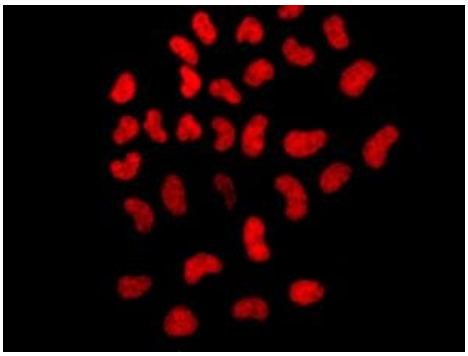
Predicted band size: 88 kDa



Immunocytochemistry/ Immunofluorescence - Anti-BRD2 antibody [EPR7642] - ChIP Grade (ab139690)

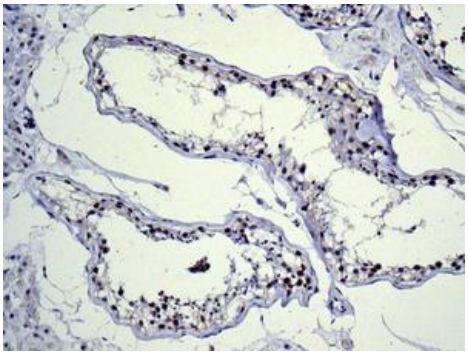
ab139690 staining Brd2 in wild-type HAP1 cells (top panel) and BRD2 knockout HAP1 cells (bottom panel). The cells were fixed with 4% formaldehyde (10min), permeabilized with 0.1% Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated with ab139690 at 0.5µg/ml 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).



Immunocytochemistry/ Immunofluorescence - Anti-BRD2 antibody [EPR7642] - ChIP Grade (ab139690)

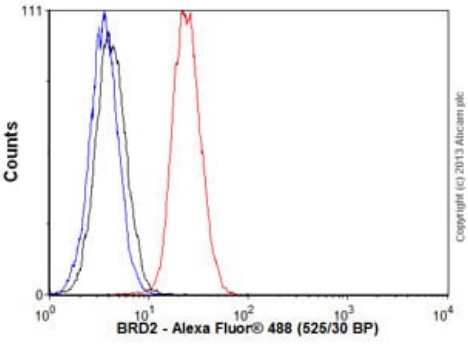
Immunofluorescence analysis of HeLa cells labeling BRD2, with ab139690 at 1/250 dilution.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-BRD2 antibody [EPR7642] - ChIP Grade (ab139690)

Immunohistochemical analysis of paraffin-embedded Human testis tissue labeling BRD2 with ab139690 at 1/250 dilution.

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



Flow Cytometry (Intracellular) - Anti-BRD2 antibody [EPR7642] - ChIP Grade (ab139690)

Overlay histogram showing HeLa cells stained with ab139690 (red line). The cells were fixed with 80% methanol (5 min) and then permeabilized with 0.1% PBS-Tween for 20 min. The cells were then incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions followed by the antibody (ab139690, 1/1000 dilution) for 30 min at 22°C. The secondary antibody used was Alexa Fluor® 488 goat anti-rabbit IgG (H&L) (**ab150077**) at 1/2000 dilution for 30 min at 22°C. Isotype control antibody (black line) was rabbit IgG (monoclonal) (0.1µg/1x10^6 cells) used under the same conditions. Unlabelled sample (blue line) was also used as a control. Acquisition of >5,000 events were collected using a 20mW Argon ion laser (488nm) and 525/30 bandpass filter.

Anti-BRD2 antibody [EPR7642] - ChIP Grade

(ab139690)

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