abcam

ab50818

Anti-BRD3 antibody [2088C3a]

KO Validated

Key facts

Isotype	IgG1
Host species	Mouse
Storage buffer	pH: 7.4 Preservative: 0.05% Sodium azide Constituents: PBS, 1% BSA
Form	Liquid
Clonality	Monoclonal
Immunogen	Recombinant Fragment Protein within Human BRD3. The exact immunogen used to generate this antibody is proprietary information. Database link Q15059
Clone number	2088C3a
Purification technique	Affinity purification Protein G
Concentration	0.1 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →
Purification notes	Purified from TCS

Reactivity data

IP

Tested

Species	Human
Dilution info	1-5 µg/mg of lysate
Notes	-

Expected

Species	Mouse, Rat
Dilution info	Use at an assay dependent concentration.
Notes	-

Flow Cyt

Tested

Species	Human
Dilution info	0.5 µg/mL
Notes	ab170190 - Mouse monoclonal IgG1, is suitable for use as an isotype control with this antibody.

Expected

Species	Mouse, Rat
Dilution info	Use at an assay dependent concentration.
Notes	-

WB

Tested

Species	Mouse
Dilution info	1/50
Notes	-

Species	Rat
Dilution info	1/50
Notes	-

Species	Human
Dilution info	1/50
Notes	-

ICC/IF

Tested

Species	Human
Dilution info	1/10
Notes	-

Expected

Species	Mouse, Rat
Dilution info	Use at an assay dependent concentration.
Notes	-

Target data

See full target information BRD3

Function

Chromatin reader that recognizes and binds acetylated histones, thereby controlling gene expression and remodeling chromatin structures (PubMed:18406326, PubMed:22464331, PubMed:27105114, PubMed:32895492). Recruits transcription factors and coactivators to target gene sites, and activates RNA polymerase II machinery for transcriptional elongation (PubMed:29567837, PubMed:32895492). In vitro, binds acetylated lysine residues on the N-terminus of histone H2A, H2B, H3 and H4 (PubMed:18406326). Involved in endoderm differentiation via its association with long non-coding RNA (lncRNA) DIGIT: BRD3 undergoes liquid-liquid phase separation upon binding to lncRNA DIGIT, promoting binding to histone H3 acetylated at 'Lys-18' (H3K18ac) to induce endoderm gene expression (PubMed:32895492). Also binds non-histones acetylated proteins, such as GATA1 and GATA2: regulates transcription by promoting the binding of the transcription factor GATA1 to its targets (By similarity).

Storage

Shipped at conditions	Blue Ice
Appropriate short-term storage duration	Up to 12 months
Appropriate short-term storage conditions	+4°C

Appropriate long-term storage conditions	-20°C

Notes

This product was changed from ascites to tissue culture supernatant on 9th August 2018. Please note that the dilutions may need to be adjusted accordingly. If you have any questions, please do not hesitate to contact our scientific support team.

Product promise

Tested

We have tested this species and application combination and it works. It is covered by our product promise.

Expected

We have not tested this specific species and application combination in-house, but expect it will work. It is covered by our product promise.

Predicted

This species and application combination has not been tested, but we predict it will work based on strong homology. However, this combination is not covered by our product promise.

Not recommended

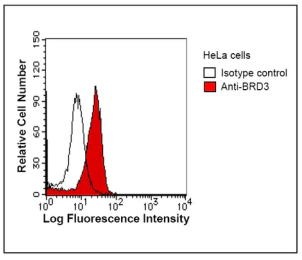
We do not recommend this combination. It is not covered by our product promise.

We are dedicated to supporting your work with high quality reagents and we are here for you every step of the way should you need us.

In the unlikely event of one of our products not working as expected, you are covered by our product promise.

Full details and terms and conditions can be found here:
Terms & Conditions.

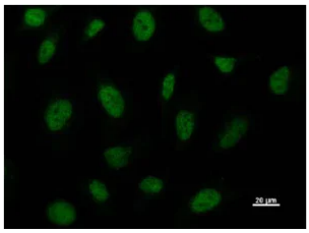
11 product images



Flow Cytometry - Anti-BRD3 antibody [2088C3a] (ab50818)

BRD3 Flow Cytometry staining using mouse Anti-BRD3 antibody

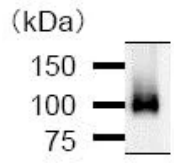
Flow Cytometric analysis using ab50818 (0.5ug). Secondary antibody used: AlexaFluor®488 Goat Anti-mouse IgG (0.25 µg)



Immunocytochemistry/ Immunofluorescence - Anti-BRD3 antibody [2088C3a] (ab50818)

BRD3 Immunocytochemistry/ Immunofluorescence staining using mouse Anti-BRD3 antibody

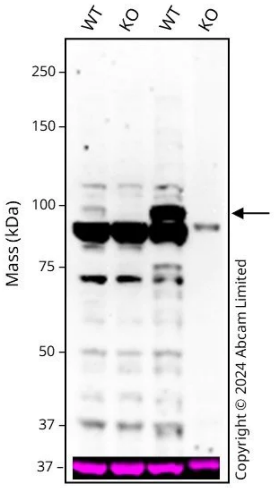
Immunoflourescent staining of Hela cells using ab50818 Anti-BRD3 Antibody (1/10 dilution) as primary and AlexaFluor®488 Goat Anti-mouse IgG(1/200 dilution)



Immunoprecipitation - Anti-BRD3 antibody [2088C3a] (ab50818)

Immunoprecipitation of BRD3 from RIPA lysate of HeLa cells (500µg) using 5µg ab50818. Visualized by western blot using 1/50 ab50518 as primary and anti-mouse IgG secondary (1:2500). Predicted band size 80 KDa.
All lanes:
Immunoprecipitation - Anti-BRD3 antibody [2088C3a] (ab50818)

Predicted band size: 80 kDa



Western blot - Anti-BRD3 antibody [2088C3a] (ab50818)

Western blot: Anti-BRD3 antibody [2088C3a] (ab50818) staining at 1/200 dilution, shown in green; Rabbit Anti-GAPDH antibody [EPR16891] (ab181602) loading control staining at 1/20000 dilution, shown in magenta. In Western blot, ab50818 was shown to bind specifically to BRD3. A band was observed at 90-100 kDa in wild-type HCT 116 cell lysates with no signal observed at this size in BRD3 knockout cell line. To generate this image, wild-type and BRD3 knockout HCT 116 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 HRP conjugated Goat anti-Mouse (H+L) and Goat anti-Rabbit IgG H&L 680RD at 1/20000 dilution.
All lanes:
Western blot - Anti-BRD3 antibody [2088C3a] (ab50818) at 1/200 dilution

Lane 1:
Wild-type HCT 116 cell lysate at 20 µg

Lane 2:
BRD3 knockout HCT 116 cell lysate at 20 µg

Lane 3:
Wild-type HEK-293T ab255553 cell lysate at 20 µg

Lane 4:
BRD3 knockout HEK-293T ab260529 cell lysate at 20 µg

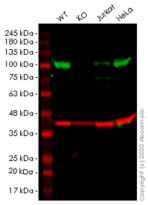
Secondary

Lanes 1 - 4:
HRP conjugated Goat anti-Mouse (H+L) at 1/20000 dilution

Lanes 1 - 4:
Goat anti-Rabbit IgG H&L 680RD at 1/20000 dilution

Performed under reducing conditions.

Observed band size: 90-100 kDa



Western blot - Anti-BRD3 antibody [2088C3a] (ab50818)

BRD3 Western blot staining using mouse Anti-BRD3 antibody

Lanes 1-4: Merged signal (red and green). Green - ab50818 observed at 100 kDa. Red - loading control ab181602 observed at 36 kDa.
ab50818 Anti-BRD3 antibody [2088C3a] was shown to specifically react with BRD3 in wild-type HEK293T cells. Loss of signal was observed when knockout cell line ab266793 (knockout cell lysate ab258335) was used. Wild-type and BRD3 knockout samples were subjected to SDS-PAGE. ab50818 and Anti-GAPDH antibody[EPR16891] - Loading Control (ab181602) were incubated overnight at 4°C at 1 in 500 dilution and 1 in 20000 dilution respectively. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 680RD) preadsorbed (ab216777) and Goat anti-Mouse IgG H&L (IRDye® 800CW) preadsorbed (ab216772) secondary antibodies at 1 in 20000 dilution for 1 hour at room temperature before imaging.

All lanes:
Western blot - Anti-BRD3 antibody [2088C3a] (ab50818) at 1/500 dilution

Lane 1:
Wild-type HEK293T cell lysate at 20 µg

Lane 2:
Western blot - Human BRD3 knockout HEK-293T cell lysate (ab258335) at 20 µg

Lane 3:
Jurkat cell lysate at 20 µg

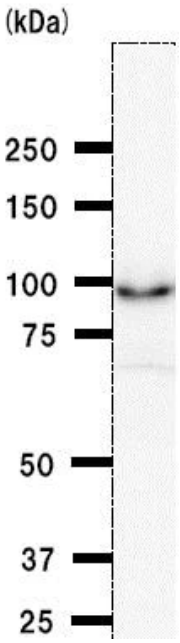
Lane 4:
HeLa cell lysate at 20 µg

Secondary

All lanes:
Western blot - Goat Anti-Rabbit IgG H&L (IRDye® 680RD) preadsorbed (ab216777) at 1/10000 dilution

Predicted band size: 80 kDa

Observed band size: 100 kDa



Western blot - Anti-BRD3 antibody [2088C3a] (ab50818)

All lanes:
Western blot - Anti-BRD3 antibody [2088C3a] (ab50818) at 1/50 dilution

All lanes:
HeLa whole cell lysate at 50 µg

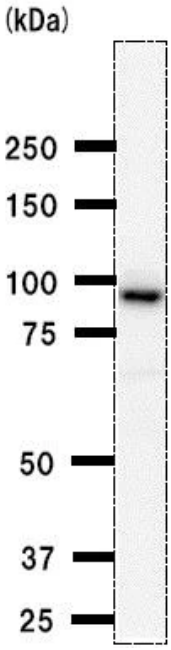
Secondary

All lanes:
Anti-Mouse IgG antibody at 1/2500 dilution

Developed using the ECL technique.

Predicted band size: 80 kDa

Observed band size: 90 kDa



Western blot - Anti-BRD3 antibody [2088C3a] (ab50818)

All lanes:
Western blot - Anti-BRD3 antibody [2088C3a] (ab50818) at 1/50 dilution

All lanes:
NIH3T3 whole cell lysate at 50 µg

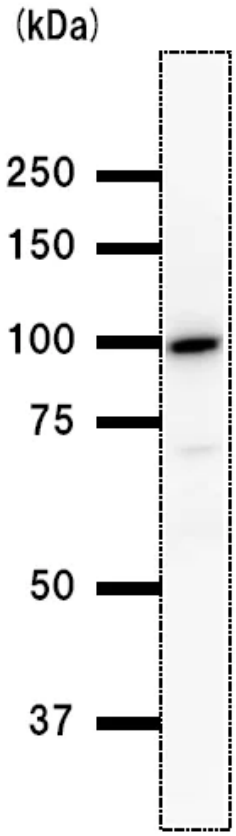
Secondary

All lanes:
Anti-Mouse IgG antibody at 1/2500 dilution

Developed using the ECL technique.

Predicted band size: 80 kDa

Observed band size: 90 kDa



Western blot - Anti-BRD3 antibody [2088C3a] (ab50818)

All lanes:
Western blot - Anti-BRD3 antibody [2088C3a] (ab50818) at 1/200 dilution

All lanes:
F2408 whole cell lysate at 50 µg

Secondary

All lanes:
Anti Mouse IgG antibody at 1/2500 dilution

Predicted band size: 80 kDa

Observed band size: 100 kDa

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e

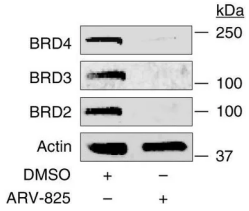


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Western blot - Anti-BRD3 antibody [2088C3a] (ab50818)

BRD3 Western blot staining using mouse Anti-BRD3 antibody

Western Blotting using Anti-BRD3 antibody [2088C3a], ab50818. Publication image from Lam, F. C. et al., 2020, Nat Commun, 32796829. Legend direct from paper.

BRD4 bromodomain inhibition causes DNA damage and apoptosis.a Left panel: Immuno-fluorescence (IF) images of γH2AX fluorescence in HeLa cells following treatment with DMSO or 500 nM JQ1 for 12 h (n = 3 independent experiments). Significance assessed using two-tailed unpaired t test (**P = 0.0058). b Fluorescence microscopy images of neutral comet single cell gel electrophoresis assay in cells treated for 12 h with DMSO or JQ1 (n = 3). Box-whisker plots indicate median, 25th to 75th percentile, and maximum and minimum values by line, box, and whiskers, respectively. Significance assessed using two-tailed unpaired t test (****P < 0.0001). c Left panel: lysates from control or JQ1-treated cells were analyzed by western blotting (WB) for γH2AX and cleaved PARP (cPARP). Actin or tubulin serves as loading controls in this and subsequent WB. Right panel: Relative γH2AX and cPARP band intensities were quantified (n = 3). Significance assessed using one-tailed unpaired t test (*PγH2AX = 0.0347, ***PcPARP = 0.0002). d HeLa cells were treated with either DMSO or JQ1 and their growth plotted over a course of 72 h (n = 3, error bars indicate SEM). e HeLa cells were treated with the BET bromodomain proteasome-targeting small molecule ARV-825 (100 nM) for 3 h, and lysates probed for levels of BRD2, BRD3, and BRD4 by WB. f Representative IF images of γH2AX foci in cells treated with ARV-825 for 6 h (n = 3). Significance assessed using one-tailed unpaired t test (**P = 0.0011). g Fluorescence microscopy images of neutral cell single cell gel electrophoresis assay of cells treated with ARV-825 for 6 h (n = 3 independent experiments). Box-whisker plots drawn as in b. Significance assessed using two-tailed unpaired t test (****P < 0.0001). g Lysates from cells treated with ARV-825 (100 nM for 6 h) were analyzed for γH2AX, cPARP and actin by WB. Intensities of the γH2AX and cPARP bands were quantified (n = 3). Significance assessed using two-tailed unpaired t test (***PγH2AX = 0.0009, ***PcPARP = 0.0001). In panels a, c, f, and h data are presented as mean ± SEM. Scale bars in a, f = 5 µm. Source data are provided as a Source data file.

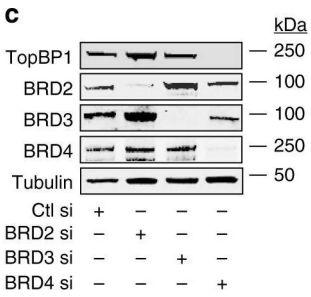


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BRD4 loss impairs the replication stress DNA damage checkpoint.a Overview of the ATR-TopBP1-Chk1 replication stress DNA damage checkpoint. TopBP1 is required for Chk1 activation and recruitment31,82. b Quantification of RT-qPCR of ATRIP, ATR, TopBP1, and Chk1 mRNA levels following treatment with 500 nM JQ1 for 24 and 48 h in HeLa cells. Data presented as mean ± SEM (n = 3 independent experiments). Significance assessed using ANOVA followed by Tukey's test (24 h DMSOTopBP1 vs JQ1TopBP1 ****Adjusted P < 0.0001; 48 h DMSOTopBP1 vs JQ1TopBP1 ****Adjusted P < 0.0001). c WB of TopBP1, BRD2, BRD3, BRD4, and tubulin following transfection with control siRNA or siRNA to BRD2, BRD3, or BRD4. d, e WB of pATRthr1989, ATR, pTopBP1ser1138, TopBP1, and tubulin (d), and pChk1ser345, Chk1, and tubulin (e), following 24 h of treatment with JQ1. f IF images and quantification as in Fig. 1a of cells that stain triply positive for γH2AX, CC3 and EdU (upper panel) or γH2AX, CC3, and pHH3 (lower panel) following JQ1 treatment. Data presented as mean ± SEM (n = 3 independent experiments). Significance assessed using two-tailed unpaired t test (24 h DMSOγH2AX/CC3/EdU (+) vs JQ1γH2AX/CC3/EdU (+) ****P < 0.0001; 48 h DMSOγH2AX/CC3/EdU (+) vs JQ1γH2AX/CC3/EdU (+) ****P < 0.0001; 48 h DMSOγH2AX/CC3/pHH3 (+) vs JQ1γH2AX/CC3/pHH3 (+) **P = 0.0012). Scale bar = 5 µm. g Global model of how BRD4 loss results in DNA damage and cell death as a consequence of loss of productive RNAPII elongation, R-loop accumulation, transcription-replication conflicts, and failure to activate the ATR-TopBP1-Chk1 axis. Source data are provided as a Source data file.

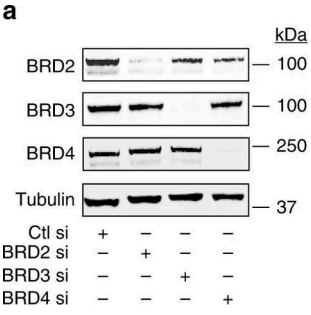


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Western blot - Anti-BRD3 antibody [2088C3a] (ab50818)

BRD3 Western blot staining using mouse Anti-BRD3 antibody

Western Blotting using Anti-BRD3 antibody [2088C3a], ab50818. Publication image from Lam, F. C. et al., 2020, Nat Commun, 32796829. Legend direct from paper.

Specific loss of BRD4 leads to R-loop-induced DNA damage.a WB of BRD2, BRD3, and BRD4 following transfection of HeLa cells with control siRNA, or siRNAs to BRD2, BRD3, and BRD4 for 48 h. b Left: WB of γH2AX, total PARP, and total H2AX in cells following siRNA knockdown of BRD2, BRD3, or BRD4 in HeLa cells. Right: Quantification of γH2AX and cleaved PARP product (cPARP; lower band on PARP western blot) signal following knockdown of BRD2, BRD3, or BRD4. Data presented as mean ± SEM (n = 3 independent experiments). Significance assessed using ANOVA followed by Sidak's test (γH2AXCtl si vs BRD4 si **Adjusted P = 0.0080; cPARPCtl si vs BRD4 si *Adjusted P = 0.0254). c Quantification and representative IF images of γH2AX and nuclear S9.6 intensities in cells following siRNA knockdown of BRD2, BRD3, or BRD4. Data presented as mean ± SEM (n = 3 separate experiments). Box-whisker plots are drawn as in Fig. 1b. Significance was assessed using ANOVA followed by Dunnett's test (γH2AXCtl si vs BRD4 si ***Adjusted P = 0.0004; S9.6Ctl si vs BRD4 si ****Adjusted P < 0.0001). Scale bar = 2.5 µm. d HeLa cells expressing doxycycline-inducible FLAG-tagged RNase H1 were treated with doxycycline for 24 h then were transfected with control or BRD4 siRNA for 48 h. Lysates were probed for BRD4 and FLAG-tagged RNase H1 by WB. Tubulin serves as loading control. e Fluorescence microscopy images of single cell gel electrophoresis showing tail moments following knockdown of BRD4 (BRD4 si) in the absence or presence of RNase H1 induction (n = 3). Box-whisker plots are drawn as in Fig. 1b. Significance was assessed using ANOVA followed by Tukey's test ****Adjusted P < 0.0001, **Adjusted P = 0.0069. d, e Are from the same experiment. f Representative WB of BRD4, FLAG-tagged RNase H1, and γH2AX following knockdown of BRD4 and doxycycline-induced expression of FLAG-tagged RNase H1. Quantification of γH2AX intensity presented as mean (n = 2 independent experiments).g Cell counts of RNase H-inducible HeLa cells following siRNA knockdown of BRD2, BRD3, or BRD4 for 72 h, in the presence (+RNase H, red line) or absence (−RNase H, blue line) of induced RNase H expression (n = 2 independent experiments). Source data are provided as a Source data file.

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