abcam

ab243862

Anti-Brd4 antibody [BL-149-2H5]

Anti-Brd4 antibody [BL-149-2H5] (ab243862) is a rabbit monoclonal antibody detecting Brd4 in **Western Blot, Flow Cytometry, IP, IHC-P, ICC/IF, ChIP-seq**. Suitable for **Human, Mouse**.

- Recombinant format for unrivalled batch-batch consistency

Lab Essentials | Recombinant | Advanced Validation

Key facts

Isotype	IgG
Host species	Rabbit
Storage buffer	pH: 7.8 - 8.6 Preservative: 0.09% Sodium azide Constituents: 98% Borate buffered saline, 0.1% BSA
Form	Liquid
Clonality	Monoclonal
Immunogen	Synthetic Peptide within Human BRD4 aa 1300 to C-terminus. The exact immunogen used to generate this antibody is proprietary information. Database link O60885
Clone number	BL-149-2H5
Concentration	1 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →
Purification notes	Recombinant antibody was purified from cell culture supernatant.

Reactivity data

IHC-P

Tested

Species	Human

Dilution info	1/100 - 1/500
Notes	Perform heat-mediated antigen retrieval with citrate buffer pH 6 before commencing with IHC staining protocol.

Expected

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

IP

Tested

Species	Human
Dilution info	-
Notes	Use 5-10µl/0.5mg lysate.

Expected

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

Flow Cyt

Tested

Species	Human
Dilution info	1 µL for 10^6 Cells
Notes	-

Expected

Species	Mouse
Dilution info	Use at an assay dependent concentration.

Notes	-

WB

Tested

Species	Mouse
Dilution info	1/1000
Notes	-

Species	Human
Dilution info	1/1000
Notes	-

ICC/IF

Tested

Species	Human
Dilution info	1/100 - 1/500
Notes	-

Expected

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

ChIP-seq

Tested

Species	Human
Dilution info	10-40 µL for 30 µL chromatin
Notes	-

Expected

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

Target data

See full target information BRD4

Function	Chromatin reader protein that recognizes and binds acetylated histones and plays a key role in transmission of epigenetic memory across cell divisions and transcription regulation (PubMed:20871596, PubMed:23086925, PubMed:23317504, PubMed:29176719, PubMed:29379197). Remains associated with acetylated chromatin throughout the entire cell cycle and provides epigenetic memory for postmitotic G1 gene transcription by preserving acetylated chromatin status and maintaining high-order chromatin structure (PubMed:22334664, PubMed:23317504, PubMed:23589332). During interphase, plays a key role in regulating the transcription of signal-inducible genes by associating with the P-TEFb complex and recruiting it to promoters (PubMed:16109376, PubMed:16109377, PubMed:19596240, PubMed:23589332, PubMed:24360279). Also recruits P-TEFb complex to distal enhancers, so called anti-pause enhancers in collaboration with JMJD6 (PubMed:16109376, PubMed:16109377, PubMed:19596240, PubMed:23589332, PubMed:24360279). BRD4 and JMJD6 are required to form the transcriptionally active P-TEFb complex by displacing negative regulators such as HEXIM1 and 7SKsnRNA complex from P-TEFb, thereby transforming it into an active form that can then phosphorylate the C-terminal domain (CTD) of RNA polymerase II (PubMed:16109376, PubMed:16109377, PubMed:19596240, PubMed:23589332, PubMed:24360279). Regulates differentiation of naive CD4(+) T-cells into T-helper Th17 by promoting recruitment of P-TEFb to promoters (By similarity). Promotes phosphorylation of 'Ser-2' of the C-terminal domain (CTD) of RNA polymerase II (PubMed:23086925). According to a report, directly acts as an atypical protein kinase and mediates phosphorylation of 'Ser-2' of the C-terminal domain (CTD) of RNA polymerase II; these data however need additional evidences in vivo (PubMed:22509028). In addition to acetylated histones, also recognizes and binds acetylated RELA, leading to further recruitment of the P-TEFb complex and subsequent activation of NF-kappa-B (PubMed:19103749). Also acts as a regulator of p53/TP53-mediated transcription: following phosphorylation by CK2, recruited to p53/TP53 specific target promoters (PubMed:23317504). Isoform B. Acts as a chromatin insulator in the DNA damage response pathway. Inhibits DNA damage response signaling by recruiting the condensin-2 complex to acetylated histones, leading to chromatin structure remodeling, insulating the region from DNA damage response by limiting spreading of histone H2AX/H2A.x phosphorylation.

Storage

Shipped at conditions	Blue Ice

Appropriate short-term storage duration	1-2 weeks
Appropriate short-term storage conditions	+4°C
Appropriate long-term storage conditions	+4°C
Aliquoting information	Upon delivery aliquot
Storage information	Avoid freeze / thaw cycle

Notes

What is this antibody validated in?
Anti-Brd4 antibody [BL-149-2H5] (ab243862) is a rabbit recombinant monoclonal antibody and is validated for use in Western Blot (WB), Flow Cytometry (Flow Cyt), Immunoprecipitation (IP), Immunohistochemistry (IHC-P), Immunocytochemistry/immunofluorescence (ICC/IF), ChIP in Human, Mouse samples.

Other related products
We have a range of other formats of antibody clone [BL-149-2H5] also available for your convenience: ab243862, ab272042

REACH authorisation
Abcam has not and does not intend to apply for the REACH Authorisation of customers' uses of products that contain European Authorisation list (Annex XIV) substances.
It is the responsibility of our customers to check the necessity of application of REACH Authorisation, and any other relevant authorisations, for their intended uses.

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Supplementary info

This supplementary information is collated from multiple sources and compiled automatically.

Activity summary	The Brd4 protein also known as Bromodomain containing protein 4 plays an important role as a transcriptional regulator. It recognizes acetylated lysine residues on histone tails through its bromodomains. This binding impacts chromatin structure and transcriptional activation. The Brd4 protein has a molecular weight of around 150 kDa and is often used as a marker in studies using Brd4 western blot techniques. It mainly expresses in the nucleus of most cell types and is an important focus for developing Brd4 anticuerpos useful for detecting protein size discrepancies in research.
Biological function summary	Brd4 plays a significant role in regulating gene expression during cell cycle progression. It interacts with the Mediator complex enabling communication between transcriptional activators and RNA polymerase II. This protein is integral for maintaining chromatin in an active state ensuring accessibility for transcription

	machinery. Moreover Brd4 size and functionality relate to the elongation of transcription by RNA polymerase II ensuring the seamless expression of genes essential for cellular functions.
Pathways	Brd4 integrates into transcriptional and epigenetic regulation pathways. It acts in the positive regulation of the MYC pathway directly linking Brd4 with proto-oncogene c-Myc. Also Brd4 engages in the JAK/STAT signaling pathway where its activity affects cell growth and immune response. These interactions establish Brd4 as a central regulator within these pathways facilitating communication and coordination among molecular players.
Associated diseases and disorders	Brd4 link heavily to cancer and inflammatory diseases. Aberrant Brd4 activity often associates with various cancers including leukemia where its regulation impacts oncogenic drivers. Clinically targeting Brd4 presents a therapeutic avenue as its inhibition affects cyclin-dependent kinase 9 (CDK9) an important regulator of the cell cycle. Similarly Brd4 alterations implicate in inflammatory disorders impacting nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kB) signaling revealing pathways for intervening in pathogenic processes.

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

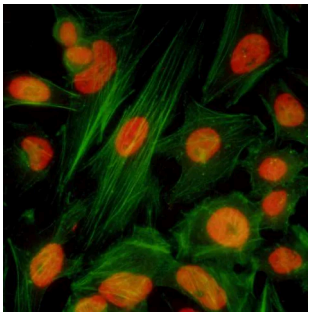
We do not recommend this combination. It is not covered by our product promise.

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.

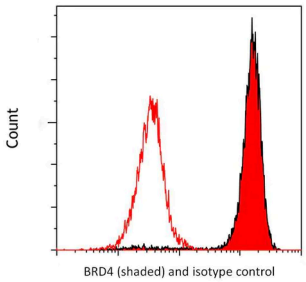
11 product images



Immunocytochemistry/ Immunofluorescence - Anti-Brd4 antibody [BL-149-2H5] (ab243862)

Brd4 Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-Brd4 antibody

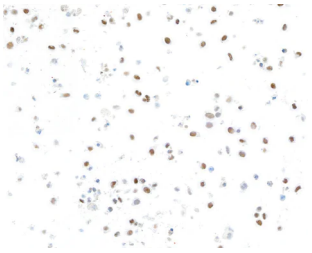
Formaldehyde-fixed HeLa (human epithelial cell line from cervix adenocarcinoma) cells labeling brd4 using ab243862 at 1/250 dilution in ICC/IF analysis. DyLight® 594-conjugated goat anti-rabbit IgG was used as the secondary antibody.



Flow Cytometry - Anti-Brd4 antibody [BL-149-2H5] (ab243862)

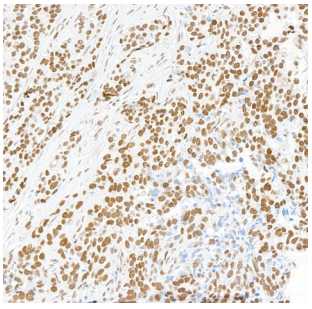
Brd4 Flow Cytometry staining using rabbit Anti-Brd4 antibody

Flow cytometric analysis of 4% formaldehyde and permeabilized with 90% methanol KG-1 (Human acute myeloid leukemia cell line from bone marrow) cells labelling BRD4 with ab243862 at 1µl/10^6 cells (Red shaded) compared with a Isotype control (unshaded). DyLight® 650-conjugated goat anti-rabbit IgG was used as the secondary antibody.



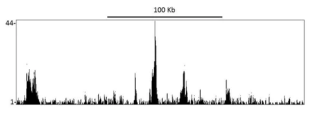
Immunocytochemistry - Anti-Brd4 antibody [BL-149-2H5] (ab243862)

Immunocytochemical analysis of Formalin-Fixed Paraffin-Embedded HeLa cells labeling human BRD4 with ab243862, followed by a HRP-conjugated goat anti-rabbit IgG as secondary. Substrate: DAB. Nuclear staining on HeLa cells is observed. Counter stained with hematoxylin. Epitope retrieval with citrate buffer pH 6.0 for 20 minutes is recommended.



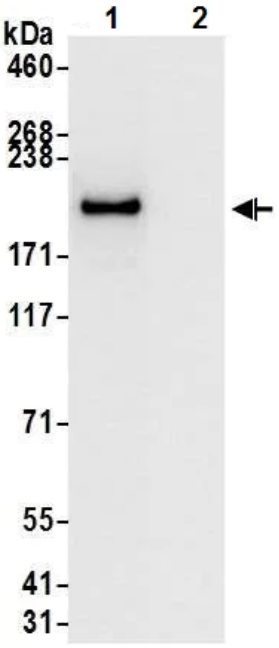
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Brd4 antibody [BL-149-2H5] (ab243862)

Immunohistochemical analysis of Formalin-Fixed Paraffin-Embedded human breast carcinoma tissue labeling BRD4 with ab243862, followed by a HRP-conjugated goat anti-rabbit IgG as secondary. Substrate: DAB. Nuclear staining on human breast carcinoma is observed. Counter stained with hematoxylin. Epitope retrieval with citrate buffer pH 6.0 for 20 minutes is recommended.



ChIP-sequencing - Anti-Brd4 antibody [BL-149-2H5] (ab243862)

Localization of human BRD4 binding sites in immunoprecipitates from Mia PaCa-2 cells by ChIP-Seq using ab243862.



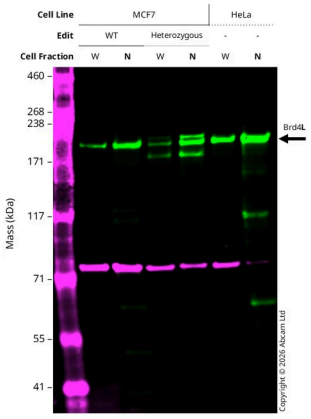
Immunoprecipitation - Anti-Brd4 antibody [BL-149-2H5] (ab243862)

brd4 was immunoprecipitated from 1 mg HeLa (Human cervix adenocarcinoma epithelial cell) whole cell lysate with ab243862 at 20 µL/mg lysate. Western blot was performed on the immunoprecipitate using ab243862 at 1/1000 dilution.
Lane 1: ab243862 IP in HeLa cell lysate.

Lane 2: Contol IgG in HeLa cell lysate.

All lanes:
Immunoprecipitation - Anti-Brd4 antibody [BL-149-2H5] (ab243862)

Predicted band size: 152 kDa



Western blot - Anti-Brd4 antibody [BL-149-2H5] (ab243862)

Brd4 Western blot staining using rabbit Anti-Brd4 antibody

Western blot: Anti-Brd4 antibody [BL-149-2H5] ab243862 staining at 1/1000 dilution, shown in green; Mouse anti-CANX ab238078 loading control staining at 1/20000 dilution, shown in magenta. A band was observed at 200 kDa for Brd4L in Wild-type MCF7 with a diminished signal detected in CRISPR-Cas9 Edited MCF7 cells expressing a heterozygous Brd4 knockout. Nuclear fractions exhibit enriched Brd4 expression in MCF7 and HeLa cell lines. To generate this image, 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 800CW and Goat anti-Mouse 680RD at 1/20,000 dilution. Abbreviations: W – Whole cell lysate; N – Nuclear fraction lysate; WT – Wild-Type.
All lanes:
Western blot - Anti-Brd4 antibody [BL-149-2H5] (ab243862) at 1/1000 dilution

Lane 1:
Human wild-type MCF7 cell lysate at 20 µg

Lane 2:
Western blot - Human BRD4 knockout MCF7 cell line (ab282674) at 20 µg

Lane 3:
BRD4 knockout MCF7 cell lysate at 20 µg

Lane 4:
BRD4 knockout MCF7 nuclear cell lysate at 20 µg

Lane 5:
HeLa cell lysate at 20 µg

Lane 6:
HeLa nuclear cell lysate at 20 µg

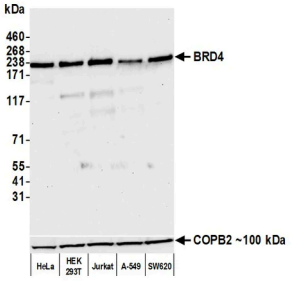
Secondary

All lanes:
Goat anti-Rabbit 800CW & Goat anti-Mouse 680RD at 1/20000 dilution

Performed under reducing conditions.

Predicted band size: 200 kDa

Observed band size: 200 kDa



Western blot - Anti-Brd4 antibody [BL-149-2H5] (ab243862)

All lanes:
Western blot - Anti-Brd4 antibody [BL-149-2H5] (ab243862) at 1/1000 dilution

Lane 1:
HeLa whole cell lysate at 25 µg

Lane 2:
HEK293T whole cell lysate at 25 µg

Lane 3:
Jurkat whole cell lysate at 25 µg

Lane 4:
A-549 whole cell lysate at 25 µg

Lane 5:
SW620 whole cell lysate at 25 µg

Secondary

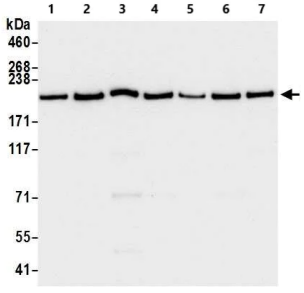
All lanes:
Western blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051)

Developed using the ECL technique.

Predicted band size: 152 kDa

Observed band size: 210 kDa

Exposure time: 10s



Western blot - Anti-Brd4 antibody [BL-149-2H5] (ab243862)

Brd4 Western blot staining using rabbit Anti-Brd4 antibody

Detection: Chemiluminescence.
Lysates prepared using NETN lysis buffer.

All lanes:
Western blot - Anti-Brd4 antibody [BL-149-2H5] (ab243862) at 1/1000 dilution

Lane 1:
HeLa whole cell lysate at 15 µg

Lane 2:
HEK293T whole cell lysate at 15 µg

Lane 3:
Jurkat whole cell lysate at 15 µg

Lane 4:

MDA-MB-231 whole cell lysate at 15 µg

Lane 5:
A-549 whole cell lysate at 15 µg

Lane 6:
SW620 whole cell lysate at 15 µg

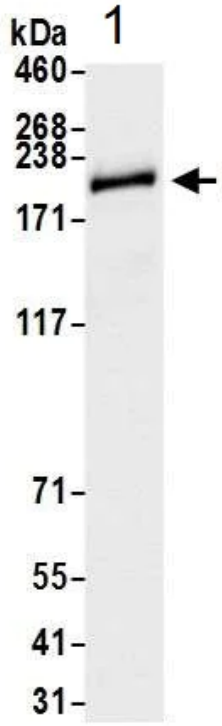
Lane 7:
SK-MEL-28 whole cell lysate at 15 µg

Secondary

All lanes:
HRP-conjugated goat anti-rabbit IgG

Predicted band size: 152 kDa

Exposure time: 3s



Western blot - Anti-Brd4 antibody [BL-149-2H5] (ab243862)

Brd4 Western blot staining of BW5147.3 (Mouse T cell lymphoma cell line from thymus) whole cell lysate using rabbit Anti-Brd4 antibody

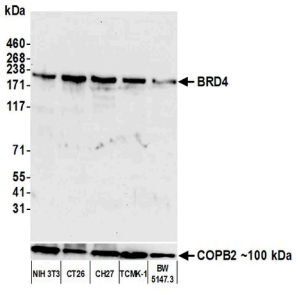
Chemiluminescence with an exposure time of 3 seconds.
All lanes:
Western blot - Anti-Brd4 antibody [BL-149-2H5] (ab243862) at 1/1000 dilution

All lanes:
BW5147.3 (Mouse T cell lymphoma cell line from thymus) whole cell lysate at 50 µg

Secondary

All lanes:
HRP-conjugated goat anti-rabbit IgG antibody

Predicted band size: 152 kDa



Western blot - Anti-Brd4 antibody [BL-149-2H5] (ab243862)

All lanes:
Western blot - Anti-Brd4 antibody [BL-149-2H5] (ab243862) at 1/1000 dilution

Lane 1:
NIH 3T3 whole cell lysate at 10 µg

Lane 2:
CT26 whole cell lysate at 10 µg

Lane 3:
CH27 whole cell lysate at 10 µg

Lane 4:
TCMK-1 whole cell lysate at 10 µg

Lane 5:
BW5147.3 whole cell lysate at 10 µg

Secondary

All lanes:
Western blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051)

Developed using the ECL technique.

Predicted band size: 155 kDa

Observed band size: 210 kDa

Exposure time: 10s

Please note: All products are 'FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC OR THERAPEUTIC PROCEDURES'.