abcam

ab128874

Anti-Brd4 antibody [EPR5150(2)]

Anti-Brd4 antibody [EPR5150(2)] (ab128874) is a rabbit monoclonal antibody detecting Brd4 in **Western Blot, Flow Cytometry (Intra), IHC-P, ICC/IF**. Suitable for **Human, Mouse** samples.

Enhanced IHC validation data – Download the data
- detailed IHC expression profiling in normal and clinically relevant cancer FFPE TMAs – seminoma, colon and stomach
- protocols for BOND RX™ and Ventana DISCOVERY ULTRA
KO validated for confirmed specificity
Over 160 publications
Biophysical QC for unrivalled batch-batch consistency

Lab Essentials | Recombinant | RabMAb | 20ul selling size

Key facts

Isotype	IgG
Host species	Rabbit
Storage buffer	pH: 7.2 - 7.4 Preservative: 0.01% Sodium azide Constituents: PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA
Form	Liquid
Clonality	Monoclonal
Immunogen	The exact immunogen used to generate this antibody is proprietary information.
Clone number	EPR5150(2)
Purification technique	Affinity purification Protein A
Dissociation constant	3.2×10^{-11} M
Concentration	0.457 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

IHC-P

Tested

Species	Human
Dilution info	1/200
Notes	**For unpurified use at 1/40** Perform heat-mediated antigen retrieval before commencing with IHC staining protocol.

Expected

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

Predicted

Species	Rat
Dilution info	-
Notes	-

WB

Tested

Species	Mouse
Dilution info	1/200 - 1/1000
Notes	-

Species	Human
Dilution info	1/200 - 1/1000
Notes	-

Predicted

Species	Rat

Dilution info	-
Notes	-

ICC/IF

Tested

Species	Human
Dilution info	1/200
Notes	**For unpurified use at 1/40**

Expected

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

Predicted

Species	Rat
Dilution info	-
Notes	-

Flow Cyt (Intra)

Tested

Species	Human
Dilution info	1/50
Notes	-

Expected

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

Predicted

Species	Rat
Dilution info	-
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	Conditional Ambient
Appropriate short-term storage duration	1-2 weeks
Appropriate short-term storage conditions	+4°C
Appropriate long-term storage conditions	-20°C
Aliquoting information	Upon delivery aliquot
Storage information	Avoid freeze / thaw cycle

Notes

Anti-Brd4 antibody [EPR5150(2)] (ab128874) was developed by Abcam using patented rabbit monoclonal antibody technology and is validated for use in Flow Cyt (Intra), ICC/IF, IHC-P and WB.

Anti-Brd4 antibody [EPR5150(2)] (ab128874) was first used in a scientific publication in 2014 and has been cited over 163 times in peer reviewed journals. It's performance in Western Blot in human samples is trusted by the scientific community.

Abcam's high quality manufacturing and validation processes ensure Anti-Brd4 antibody [EPR5150(2)] (ab128874) has high sensitivity and specificity alongside high lot-to-lot consistency and reproducibility.

The specificity of Anti-Brd4 antibody [EPR5150(2)] (ab128874) has been confirmed by Western Blot testing in Brd4 knockout Hap1 cells.

Anti-Brd4 antibody [EPR5150(2)] (ab128874) has 17 independent reviews from customers.

Anti-Brd4 antibody [EPR5150(2)] (ab128874) specifically detects Brd4 (UniProt ID: O60885; Molecular weight: 153kDa) and is sold in 100 µL and 1 mL selling sizes.

Conjugation-ready, carrier free format available for antibody clone EPR5150(2) - ab182446.

Antibody clone EPR5150(2) is also available pre-conjugated to a variety of labels for your convenience - Alexa Fluor® 488, Alexa Fluor® 647, HRP, Alexa Fluor® 594 (ab197606, ab197608, ab197609, ab207057).

Top cited antibody for this target with >200 citations. A highly specific BRD4 antibody, essential for studying bromodomain-containing protein 4, epigenetic regulation and chromatin remodeling. This antibody is crucial in cancer research, particularly in understanding BRD4's role in transcription regulation, chemoresistance and tumor progression and is widely used in studies of BRD4 inhibitors and BET proteins. Research has shown that BRD4 (and BET family proteins) may be promising therapeutic targets for various Myc-driven cancers, such as Burkitt's lymphoma and certain acute myeloid leukaemia's and is studied is a potential therapeutic target.

Patented technology
Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For

details on our patents, please refer to RabMAb® patents.

What are the advantages of a recombinant monoclonal antibody?
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 batch production

For more information, read more on recombinant antibodies.

Supplementary info

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

Activity summary	BRD4 also known as Bromodomain-containing protein 4 is a member of the BET (bromodomain and extra-terminal domain) family. This protein has a molecular weight of approximately 120 - 150 kDa often used in western blot analysis to identify its presence. It binds to acetylated lysines on histone proteins facilitating the regulation of gene expression. BRD4 is mainly expressed in the nucleus and plays an essential role in remodeling chromatin structure therefore influencing transcription processes.
Biological function summary	This protein influences various cellular activities by regulating transcriptional elongation of specific genes. BRD4 forms part of a complex with P-TEFb (Positive Transcription Elongation Factor b) which phosphorylates the C-terminal domain of RNA polymerase II facilitating transcription through chromatin. It binds to acetylated histones and non-histone proteins linking it to active transcription sites and contributing to the dynamic regulation of gene expression in response to cellular signals.
Pathways	BRD4 participates in key signaling networks like the NF-κB pathway and MYC signaling. It interacts with transcription factors and other proteins such as Cyclin T1 and CDK9 within the P-TEFb complex to regulate gene expression. BRD4 also collaborates with MYC a critical transcription factor regulating cell growth and proliferation to modulate pathways related to cellular growth and survival.
Associated diseases and disorders	BRD4 has been linked to cancer and inflammation-related conditions. Overexpression or mutations in BRD4 can lead to aberrant cell proliferation in certain cancers including breast and leukemia where it influences the expression of oncogenes. In inflammation BRD4 modulates the expression of pro-inflammatory cytokines. Through these pathways it is connected to related proteins like NF-κB which orchestrates cellular responses to inflammation and stress signals.

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.

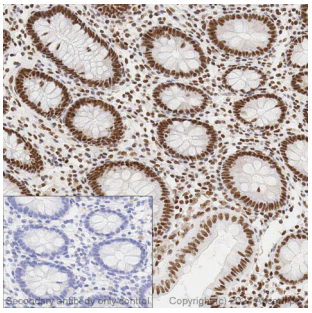
We are dedicated to supporting your work with high quality reagents and we are here for you every step of the way should you need us.

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.

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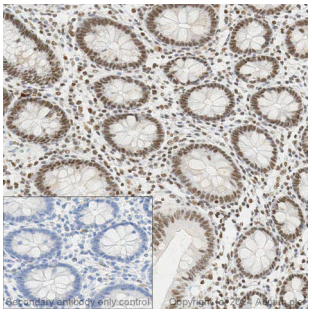
11 product images



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Brd4 antibody [EPR5150(2)] (ab128874)

Brd4 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining of human colon using rabbit Anti-Brd4 antibody

Immunohistochemical analysis of formalin fixed paraffin embedded human colon labelling Brd4 with ab128874 at a concentration of 1 µg/ml. The immunostaining was performed on a Leica Biosystems BOND® RX instrument with a Bond™ Polymer Refine Detection kit. Heat mediated antigen retrieval was performed with Tris-EDTA buffer (pH 9.0, Epitope Retrieval Solution 2) for 20 mins. ab128874 Anti-Brd4 antibody [EPR5150(2)] was incubated for 30 mins at room temperature. Sections were counterstained with Hematoxylin. Image inset shows absence of staining in secondary antibody only control.

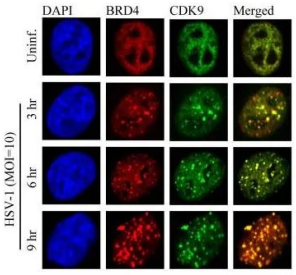


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Brd4 antibody [EPR5150(2)] (ab128874)

Brd4 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining of human colon using rabbit Anti-Brd4 antibody

Immunohistochemical analysis of formalin fixed paraffin embedded human colon labelling Brd4 with ab128874 at a concentration of 3 µg/ml. The immunostaining was performed on a Ventana DISCOVERY ULTRA (Roche Tissue Diagnostics) instrument with an OptiView DAB IHC Detection Kit. Heat mediated antigen retrieval was conducted for 32 mins at 100°C with ULTRA cell conditioning solution (CC1, pH 8.5). ab128874 Anti-Brd4 antibody [EPR5150(2)] was incubated at 37°C for 16

mins. Sections were counterstained with Hematoxylin II. Image inset shows absence of staining in secondary antibody only control.



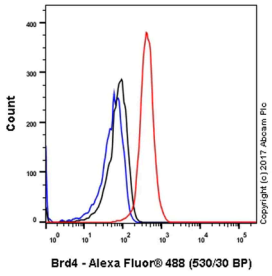
Ren et al PLoS Pathog. 2016 Oct; 12(10): e1005950. Published online 2016 Oct 20. doi: 10.1371/journal.ppat.1005950

Immunocytochemistry/ Immunofluorescence - Anti-Brd4 antibody [EPR5150(2)] (ab128874)

Brd4 Immunocytochemistry/ Immunofluorescence staining of HeLa cells using rabbit Anti-Brd4 antibody

Immunocytochemical analysis of HeLa cells showing co-localization of BRD4 using ab128874 at 1/500 dilution (red) with CDK9 (green) following infection with HSV-1. Cells were fixed with 4% paraformaldehyde (10 min at RT) and permeabilzed with 0.2% Triton X-100 (10 min). AlexaFluor®-conjugated secondary antibodies were used at 1/1000 dilution. The nuclear counter stain is DAPI 9blue).
From Figure 5b of Ren et al.

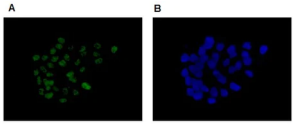
Reproduced under the Creative Commons Licence fromRen et al PLoS Pathog. 2016 Oct; 12(10): e1005950. Published online 2016 Oct 20. doi: 10.1371/journal.ppat.1005950 PMID: 27764245



Flow Cytometry (Intracellular) - Anti-Brd4 antibody [EPR5150(2)] (ab128874)

Brd4 Flow Cytometry (Intracellular) staining of SW480 (Human colorectal adenocarcinoma epithelial cell) cells using rabbit Anti-Brd4 antibody

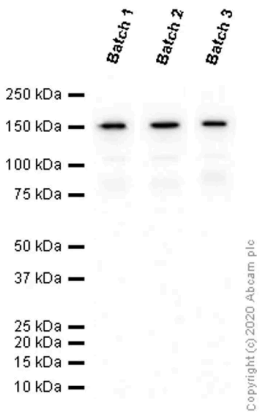
Intracellular Flow Cytometry analysis of SW480 (Human colorectal adenocarcinoma epithelial cell) cells labeling Brd4 (red) with purified ab128874 at a 1/50 dilution (10µg/mL). Cells were fixed with 80% methanol and permeabilized with 0.1% Tween-20. A goat anti rabbit IgG (Alexa Fluorr®488) (ab150077) was used as the secondary antibody at a 1/2000 dilution. Black - Rabbit monoclonal IgG (Black) (ab172730). Blue (unlabeled control) - Cell without incubation with primary antibody and secondary antibody (Blue).



Immunocytochemistry/ Immunofluorescence - Anti-Brd4 antibody [EPR5150(2)] (ab128874)

Brd4 Immunocytochemistry/ Immunofluorescence staining of HepG2 (Human liver cell line) cells using rabbit Anti-Brd4 antibody

Immunocytochemistry/Immunofluorescence analysis of HepG2 (Human liver cell line) cells labeling Brd4 with purified ab128874 at 1/100 dilution (Panel A). Cells were fixed with 4% paraformaldehyde and permeabilized with 0.1% Triton X-100. An Alexa Fluor® 488-conjugated goat anti-rabbit IgG (1/200 dilution) was used as the secondary antibody. DAPI (blue) was used as the nuclear counterstain(Panel B).

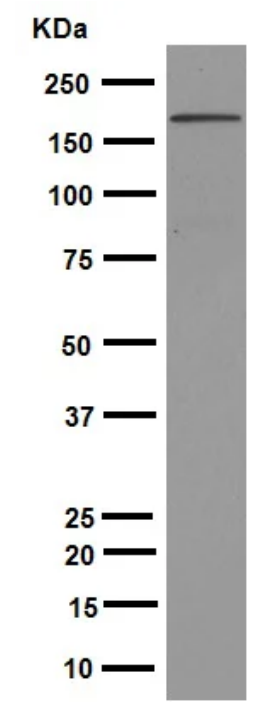


Western blot - Anti-Brd4 antibody [EPR5150(2)] (ab128874)

Brd4 Western blot staining using rabbit Anti-Brd4 antibody

Different batches of ab128874 were tested on HeLa (Human cervix adenocarcinoma epithelial cell) lysate at 0.1 µg/ml. 15 µg of lysate was loaded in each lane. Bands observed at 152 kDa.
All lanes:
Western blot - Anti-Brd4 antibody [EPR5150(2)] (ab128874)

Predicted band size: 152 kDa



Western blot - Anti-Brd4 antibody [EPR5150(2)] (ab128874)

Brd4 Western blot staining of HeLa (Human epithelial cell line from cervix adenocarcinoma) cell lysate using rabbit Anti-Brd4 antibody

Blocking and Diluting buffer and concentration: 5% NFDM/TBST.
All lanes:
Western blot - Anti-Brd4 antibody [EPR5150(2)] (ab128874) at 1/1000 dilution

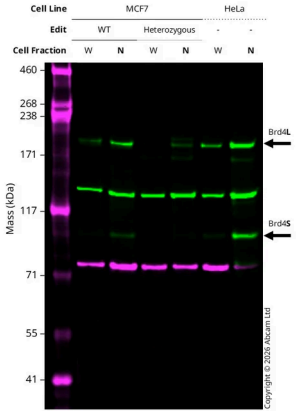
All lanes:
HeLa (Human epithelial cell line from cervix adenocarcinoma) cell lysate at 10 µg

Secondary

All lanes:
Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated at 1/1000 dilution

Predicted band size: 152 kDa

Observed band size: 152 kDa



Western blot - Anti-Brd4 antibody [EPR5150(2)] (ab128874)

Brd4 Western blot staining using rabbit Anti-Brd4 antibody

Western blot: Anti-Brd4 antibody [EPR5150(2)] ab128874 staining at 1/1000 dilution, shown in green; Anti-Calnexin antibody [CANX/1543] ab238078 loading control staining at 1/20,000 dilution, shown in magenta. A band was observed at 200 kDa for Brd4L and 100 kDa for Brd4S 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% NFDM 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 800CW and Goat anti-Mouse IgG 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 [EPR5150(2)] (ab128874) at 1/1000 dilution

Lane 1:
Wild type MCF7 (Whole cell lysate)

Lane 2:

Wild type MCF7 (Nuclear fraction lysate)

Lane 3:
Heterozygous MCF7 (Wild type)

Lane 4:
Heterozygous MCF (Nuclear fraction lysate)

Lane 5:
HeLa (whole cell lysate)

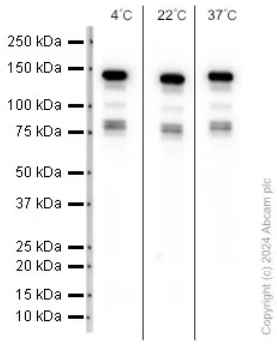
Lane 6:
HeLa (Nuclear fraction lysate)

Secondary

Lanes 1 - 6:
Goat anti-Rabbit IgG 800CW at 1/20000 dilution

Lanes 1 - 6:
Goat anti-Mouse IgG 680RD at 1/20000 dilution

Observed band size: 100 kDa, 200 kDa



Western blot - Anti-Brd4 antibody [EPR5150(2)] (ab128874)

Brd4 Western blot staining of 293T Whole cell lysate using rabbit Anti-Brd4 antibody

This blot was produced using 4-20% SDS-PAGE containing 15 µg of 293T whole cell lysate per lane at 150V for 1hr before being transferred onto a 0.45 µm PVDF membrane at 75V for 1hr. The membrane was then blocked for 1hr using 5% NFDM/TBST, then incubated with ab128874 (1/1000) at room temperature for 1hr. After being washed three times in TBST, the membrane was incubated with Peroxidase conjugated goat anti-rabbit IgG (H+L) (ab97051) at 1/20,000 dilution for 1hr at room temperature. The membrane was washed three times again. Then the signal was developed using the ECL technique.

ab128874 was stored at a range of temperatures (+4°C, +22°C, +37°C) for 1 week before being tested in WB. The image shows the band intensity remains relatively constant across all storage temperatures, demonstrating that antibody activity is not affected under these conditions. This data was generated as part of a structured antibody stability study supporting Abcam’ s transition from cold⊠chain to ambient shipping.

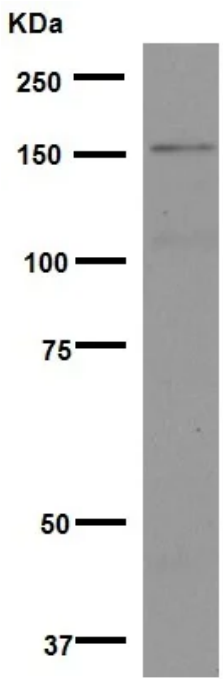
All lanes:
Western blot - Anti-Brd4 antibody [EPR5150(2)] (ab128874) at 1/1000 dilution

All lanes:
293T Whole cell lysate at 15 µg with NDFM/TBST

Secondary

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

Exposure time: 5s



Western blot - Anti-Brd4 antibody [EPR5150(2)] (ab128874)

Brd4 Western blot staining of NIH/3T3 (Mouse embryo fibroblast cell line) cell lysate using rabbit Anti-Brd4 antibody

All lanes:
Western blot - Anti-Brd4 antibody [EPR5150(2)] (ab128874) at 1/1000 dilution

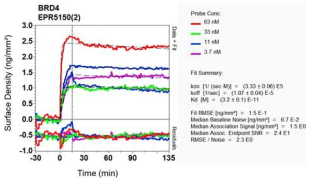
All lanes:
NIH/3T3 (Mouse embryo fibroblast cell line) cell lysate at 10 µg

Secondary

All lanes:
Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated at 1/1000 dilution

Predicted band size: 152 kDa

Observed band size: 152 kDa



OI-RD Scanning - Anti-Brd4 antibody [EPR5150(2)] (ab128874)

We have systematically measured KD (the equilibrium dissociation constant between the antibody and its antigen), of more than 840 recombinant antibodies to assess not only their individual KD values but also to see the average affinity of antibody. Based on the comparison with published literature values for mouse monoclonal antibodies, Recombinant antibodies appear to be on average 1-2 order of magnitude higher affinity.

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