abcam

ab182446

Anti-Brd4 antibody [EPR5150(2)] - BSA and Azide free

Recombinant | RabMAb

Key facts

Isotype	IgG
Host species	Rabbit
Storage buffer	pH: 7.2 Constituents: PBS
Form	Liquid
Clonality	Monoclonal
Immunogen	The exact immunogen used to generate this antibody is proprietary information.
Carrier free	Yes
Clone number	EPR5150(2)
Purification technique	Affinity purification Protein A
Dissociation constant	3.2 x 10^{-11} M
Concentration	0.993 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

IHC-P

Tested

Species	Human

Dilution info	-
Notes	-

Expected

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

Predicted

Species	Rat
Dilution info	-
Notes	-

WB

Tested

Species	Human, Mouse
Dilution info	-
Notes	-

Predicted

Species	Rat
Dilution info	-
Notes	-

ICC/IF

Tested

Species	Human
Dilution info	-
Notes	-

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	-
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	Blue Ice
Appropriate short-term storage conditions	+4°C
Appropriate long-term storage conditions	+4°C
Storage information	Do Not Freeze

Notes

ab182446 is the carrier-free version of ab128874.

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.

Conjugation ready
Our carrier-free antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. This conjugation-ready format is designed for use with fluorochromes, metal isotopes, oligonucleotides, and enzymes, which makes them ideal for antibody labelling, functional and cell-based assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications.

Use our conjugation kits for antibody conjugates that are ready-to-use in as little as 20 minutes with 1 minute hands-on-time and 100% antibody recovery: available for fluorescent dyes, HRP, biotin and gold.

Compatibility
This product is compatible with the Maxpar® Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar® is a trademark of Fluidigm Canada Inc.

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

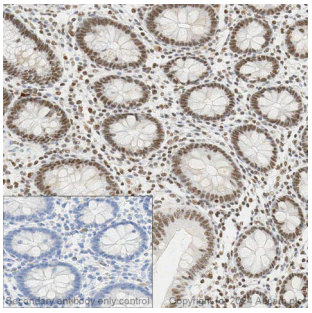
We do not recommend this combination. It is not covered by our product promise.

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.

9 product images

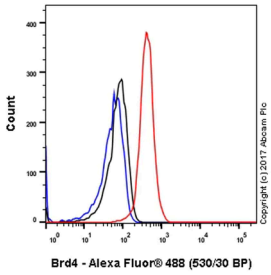


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Brd4 antibody [EPR5150(2)] - BSA and Azide free (ab182446)

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

This data was developed using the same antibody clone in a different buffer formulation (ab128874).

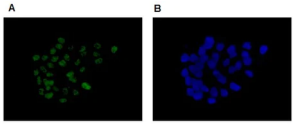
Immunohistochemical analysis of formalin fixed paraffin embedded human colon labelling Brd4 with ab128874 at a concentration of 3 µg/ml. The immunostaining was performed on a Ventana DISCOVERY ULTRA (Roche Tissue Diagnostics) instrument with an OptiView DAB IHC Detection Kit. Heat mediated antigen retrieval 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.



Flow Cytometry (Intracellular) - Anti-Brd4 antibody [EPR5150(2)] - BSA and Azide free (ab182446)

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

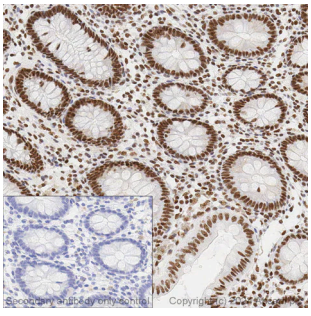
Intracellular Flow Cytometry analysis of SW480 (Human colorectal adenocarcinoma epithelial cell) cells labeling Brd4 (red) with purified ab128874 at a 1/50 dilution (10µg/mL). Cells were fixed with 80% methanol and permeabilized with 0.1% Tween-20. A goat anti rabbit IgG (Alexa Fluorr®488) (ab150077) 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).
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab128874).



Immunocytochemistry/ Immunofluorescence - Anti-Brd4 antibody [EPR5150(2)] - BSA and Azide free (ab182446)

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).
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab128874).



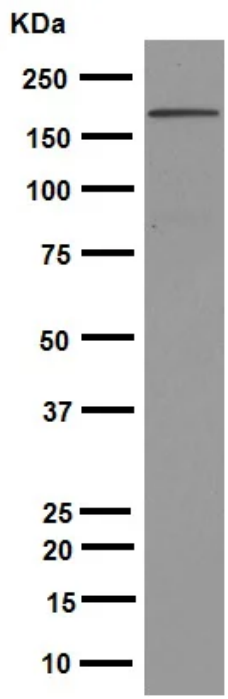
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Brd4 antibody [EPR5150(2)] - BSA and Azide free (ab182446)

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

This data was developed using the same antibody clone in a different buffer formulation (ab128874).

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.



Western blot - Anti-Brd4 antibody [EPR5150(2)] - BSA and Azide free (ab182446)

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

This data was developed using the same antibody clone in a different buffer formulation (ab128874).
Blocking and Diluting buffer and concentration: 5% NFDM/TBST.

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

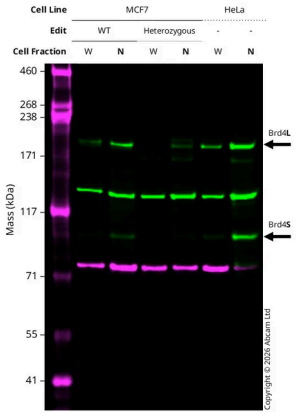
All lanes:
HeLa (Human epithelial cell line from cervix adenocarcinoma) cell lysate at 10 µg

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)] - BSA and Azide free (ab182446)

Brd4 Western blot staining using rabbit Anti-Brd4 antibody

This data was developed using ab128874, the same antibody clone in a different buffer formulation. 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)

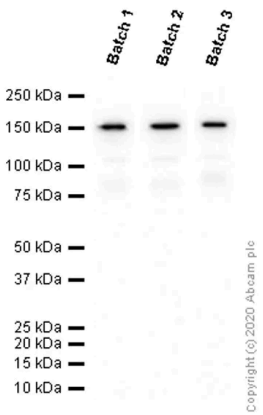
Lane 6:
HeLa (Nuclear fraction lysate)

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

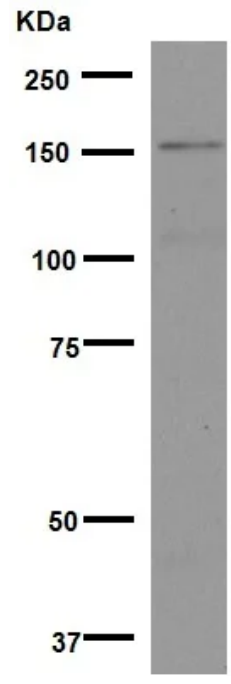


Western blot - Anti-Brd4 antibody [EPR5150(2)] - BSA and Azide free (ab182446)

Brd4 Western blot staining using rabbit Anti-Brd4 antibody

This data was developed using ab128874, the same antibody clone in a different buffer formulation. 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)] - BSA and Azide free (ab182446)

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

This data was developed using the same antibody clone in a different buffer formulation (ab128874).
All lanes:
Western blot - Anti-Brd4 antibody [EPR5150(2)] (ab128874) at 1/1000 dilution

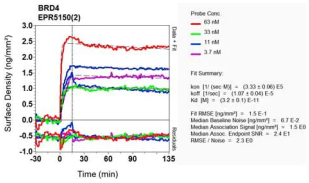
All lanes:
NIH/3T3 (Mouse embryo fibroblast cell line) cell lysate at 10 µg

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)] - BSA and Azide free (ab182446)

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'.