abcam

ab289886

# Anti-Brd4 antibody [EPR25425-23]

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** | EPR25425-23 |
| **Purification technique** | Affinity purification Protein A |
| **Specificity** | This antibody does not react with Human species for IHC. |
| **Concentration** | 0.499 mg/mL The concentration of this product may be batch-dependent<br>Batch concentration finder → |

## Reactivity data

## ICC/IF

### Tested

| | |
|---|---|
| **Species** | Mouse |
| **Dilution info** | 1/50 |
| **Notes** | - |

| | |
|---|---|
| **Species** | Human |
| **Dilution info** | 1/50 |
| **Notes** | - |

### Expected

| | |
|---|---|
| **Species** | Rat |
| **Dilution info** | Use at an assay dependent concentration. |
| **Notes** | - |

## IP

### Not recommended

| | |
|---|---|
| **Species** | Mouse, Human, Rat |
| **Dilution info** | - |
| **Notes** | - |

## ChIP

### Not recommended

| | |
|---|---|
| **Species** | Human, Mouse, Rat |
| **Dilution info** | - |
| **Notes** | - |

## IHC-P

### Tested

| | |
|---|---|
| **Species** | Mouse |
| **Dilution info** | 1/2000 |
| **Notes** | - |

| | |
|---|---|
| **Species** | Rat |
| **Dilution info** | 1/2000 |

| | |
|---|---|
| **Notes** | - |

### Not recommended

| | |
|---|---|
| **Species** | Human |
| **Dilution info** | - |
| **Notes** | - |

## Flow Cyt (Intra)

### Tested

| | |
|---|---|
| **Species** | Human |
| **Dilution info** | 1/500 |
| **Notes** | - |

| | |
|---|---|
| **Species** | Mouse |
| **Dilution info** | 1/500 |
| **Notes** | - |

### Expected

| | |
|---|---|
| **Species** | Rat |
| **Dilution info** | Use at an assay dependent concentration. |
| **Notes** | - |

## WB

### Tested

| | |
|---|---|
| **Species** | Human |
| **Dilution info** | 1/1000 |
| **Notes** | - |

| | |
|---|---|
| **Species** | Mouse |
| **Dilution info** | 1/1000 |

| | |
|---|---|
| **Notes** | - |

| | |
|---|---|
| **Species** | Rat |
| **Dilution info** | 1/1000 |
| **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

**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** | 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**

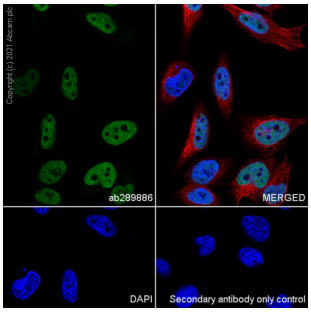
We do not recommend this combination. It is not covered by our product promise.

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.

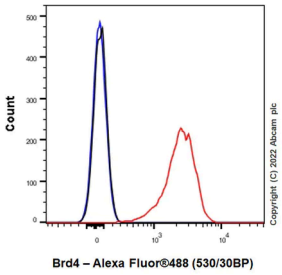
## 10 product images




## Immunocytochemistry/ Immunofluorescence - Anti-Brd4 antibody [EPR25425-23] (ab289886)

Brd4 Immunocytochemistry/ Immunofluorescence staining using rabbit Anti-Brd4 antibody

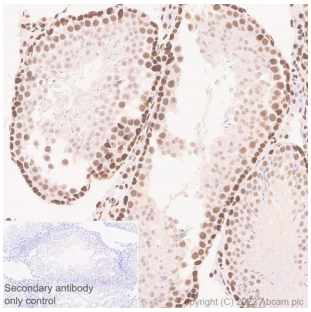
Immunofluorescent analysis of 4% Paraformaldehyde-fixed, 0.1% Triton X-100 permeabilized HeLa cells labeling Brd4 with ab289886 at 1/50 dilution, followed by ab150081 Goat Anti-Rbit IgG H&L (Alexa Fluor® 488) preadsorbed antibody at 1/1000 dilution (Green). Confocal image showing nuclear staining in HeLa cell line. ab195889 Anti-alpha Tubulin mouse monoclonal antibody - Microtubule Marker (Alexa Fluor® 594) was used to counterstain tubulin at 1/200 dilution (Red). The Nuclear counterstain was DAPI (Blue).
Secondary antibody only control: Secondary antibody is ab150081 Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed at 1/1000 dilution.




## Flow Cytometry (Intracellular) - Anti-Brd4 antibody [EPR25425-23] (ab289886)

Brd4 Flow Cytometry (Intracellular) staining using rabbit Anti-Brd4 antibody

Flow cytometric analysis of 4% paraformaldehyde fixed, 90% methanol permeabilized HeLa (Human cervix adenocarcinoma epithelial cell) cells labelling Brd4 with ab289886 at 1/500 dilution (0.1µg) (Red) compared with a Rabbit monoclonal IgG (ab172730) (Black) isotype control and an unlabelled control (cells without incubation with primary antibody and secondary antibody) (Blue). A Goat Anti-Rabbit IgG (Alexa Fluor® 488, ab150081) at 1/2000 dilution was used as the secondary antibody.

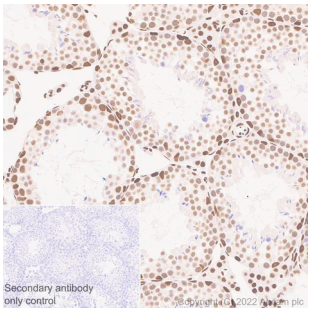



## Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Brd4 antibody [EPR25425-23] (ab289886)

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

Immunohistochemical analysis of paraffin-embedded Rat testis tissue labelling Brd4 with ab289886 at 1/2000 followed by a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101). Nucleus staining was found in rat testis. The section was incubated with ab289886 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Counterstained with Hematoxylin.
Secondary antibody only control: Secondary antibody is a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101).

Heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0, epitope retrieval solution2) for 20 mins.



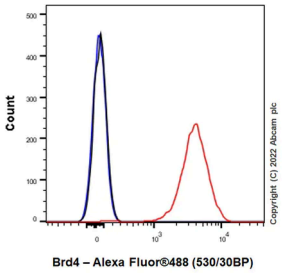

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Brd4 Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-Brd4 antibody

Immunohistochemical analysis of paraffin-embedded Mouse testis tissue labelling Brd4 with ab289886 at 1/2000 followed by a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101). Nuclear staining was found in mouse testis (PMID:28076398). The section was

incubated with ab289886 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Counterstained with Hematoxylin.
Secondary antibody only control: Secondary antibody is a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101).

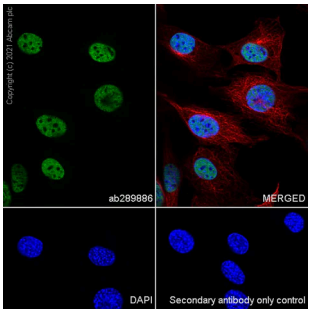
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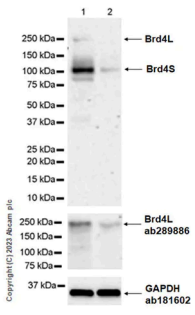
Flow cytometric analysis of 4% paraformaldehyde fixed, 90% methanol permeabilized NIH/3T3 (Mouse embryonic fibroblast) cells labelling Brd4 with ab289886 at 1/500 dilution (0.1µg) (Red) compared with a Rabbit monoclonal IgG (ab172730) (Black) isotype control and an unlabelled control (cells without incubation with primary antibody and secondary antibody) (Blue). A Goat Anti-Rabbit IgG (Alexa Fluor® 488, ab150081) at 1/2000 dilution was used as the secondary antibody.




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Secondary antibody only control: Secondary antibody is ab150081 Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed at 1/1000 dilution.




## Western blot - Anti-Brd4 antibody [EPR25425-23] (ab289886)

Blocking and diluting buffer and concentration: 5% NFDM/TBST In Western blot, Anti-GAPDH antibody [EPR16891] - Loading Control (ab181602) staining at 1/200000 dilution. In Western blot, Anti-Brd4L antibody [EPR25425-23] (ab289886) staining at 1/1000 dilution.
All lanes:
Western blot - Anti-pan Brd4 antibody [EPR25424-71] (ab314432) at 1/1000 dilution

Lane 1:
293T (human embryonic kidney epithelial cell) transfected with scrambled siRNA control whole cell lysate at 20 µg

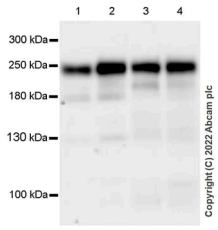
Lane 2:
293T transfected with siRNA specifically targeti Brd4 whole cell lysate at 20 µg

Secondary

All lanes:
Western blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/100000 dilution

Observed band size: 110 kDa, 200 kDa

Exposure time: 180s




## Western blot - Anti-Brd4 antibody [EPR25425-23] (ab289886)

Brd4 Western blot staining using rabbit Anti-Brd4 antibody

Blocking and diluting buffer and concentration: 5% NFDM/TBST.
Lysates were prepared from fresh material and used for Western blotting immediately to minimize protein degradation.

Exposure time: 48 seconds.

All lanes:
Western blot - Anti-Brd4 antibody [EPR25425-23] (ab289886) at 1/1000 dilution

Lane 1:
HeLa (human cervix adenocarcinoma epithelial cell) whole cell lysate at 20 µg

Lane 2:
293T (human embryonic kidney epithelial cell) whole cell lysate at 20 µg

Lane 3:
NIH/3T3 (mouse embryonic fibroblast) whole cell lysate at 20 µg

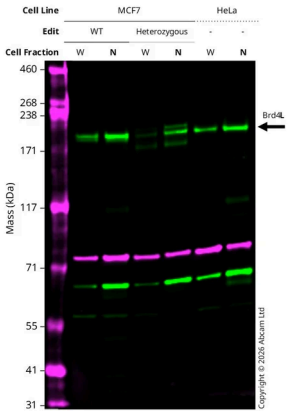
Lane 4:
PC-12 (rat adrenal gland pheochromocytoma) whole cell lysate at 20 µg

Secondary

All lanes:
Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated (ab97051) at 1/100000 dilution

Predicted band size: 152 kDa

Observed band size: 200 kDa




## Western blot - Anti-Brd4 antibody [EPR25425-23] (ab289886)

Brd4 Western blot staining using rabbit Anti-Brd4 antibody

Western blot: Anti-Brd4 antibody [EPR25425-23] ab289886 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 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 [EPR25425-23] (ab289886) 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)

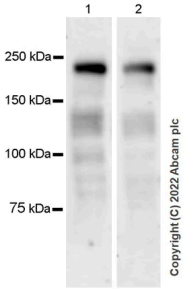
Secondary

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: 200 kDa

## Western blot - Anti-Brd4 antibody [EPR25425-23] (ab289886)




Brd4 Western blot staining using rabbit Anti-Brd4 antibody

Blocking and diluting buffer and concentration: 5% NFDM/TBST.
This blot was developed using a higher sensitivity ECL substrate.

Exposure time: 180 seconds

All lanes:
Western blot - Anti-Brd4 antibody [EPR25425-23] (ab289886) at 1/1000 dilution

Lane 1:
Mouse testis tissue lysate at 20 µg

Lane 2:
Rat testis tissue lysate at 20 µg

Secondary

All lanes:
Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated (ab97051) at 1/100000 dilution

Predicted band size: 152 kDa

Observed band size: 200 kDa

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