

## Product datasheet

# Anti-Brd4 antibody [EPR5150(2)] ab128874

**KO VALIDATED** Recombinant RabMAB

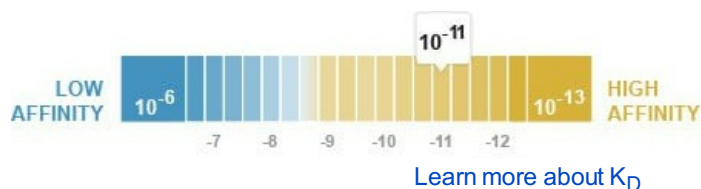
★★★★☆ 17 Abreviews 109 References 13 Images

### Overview

<b>Product name</b>	Anti-Brd4 antibody [EPR5150(2)]
<b>Description</b>	Rabbit monoclonal [EPR5150(2)] to Brd4
<b>Host species</b>	Rabbit
<b>Tested applications</b>	<b>Suitable for:</b> Flow Cyt (Intra), WB, IHC-P, ICC/IF
<b>Species reactivity</b>	<b>Reacts with:</b> Mouse, Human <b>Predicted to work with:</b> Rat
<b>Immunogen</b>	Synthetic peptide within Human Brd4 aa 150-250. The exact sequence is proprietary. Database link: <a href="#">O60885</a>
<b>Positive control</b>	WB: HeLa, Caco-2, TT, RAW 264.7 and NIH/3T3 cell lysate. Wild-type HAP1 lysate. ICC/IF: HeLa and HepG2 cells. IHC-P: Human colon carcinoma and brain tissue. Flow Cyt (intra): SW480 cells. IP: HEK-239 cell lysate.
<b>General notes</b>	<p>This product is a recombinant monoclonal antibody, which offers several advantages including:</p> <ul style="list-style-type: none"> <li>- High batch-to-batch consistency and reproducibility</li> <li>- Improved sensitivity and specificity</li> <li>- Long-term security of supply</li> <li>- Animal-free production</li> </ul> <p>For more information <a href="#">see here</a>.</p> <p>Our RabMAB<sup>®</sup> technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to <a href="#">RabMAB<sup>®</sup> patents</a>.</p> <p><b>We are constantly working hard to ensure we provide our customers with best in class antibodies. As a result of this work we are pleased to now offer this antibody in purified format. We are in the process of updating our datasheets. The purified format is designated 'PUR' on our product labels. If you have any questions regarding this update, please contact our Scientific Support team.</b></p>

### Properties

<b>Form</b>	Liquid
<b>Storage instructions</b>	Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C long term. Avoid freeze / thaw cycle.

**Dissociation constant (K<sub>D</sub>)**K<sub>D</sub> = 3.20 x 10<sup>-11</sup> M**Storage buffer**

pH: 7.20  
 Preservative: 0.01% Sodium azide  
 Constituents: PBS, 40% Glycerol, 0.05% BSA

**Purity**

Protein A purified

**Clonality**

Monoclonal

**Clone number**

EPR5150(2)

**Isotype**

IgG

**Applications****The Abpromise guarantee**Our [Abpromise guarantee](#) covers the use of ab128874 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
Flow Cyt (Intra)		1/50.
WB	★★★★★ (9)	1/200 - 1/1000. Detects a band of approximately 152 kDa (predicted molecular weight: 152 kDa).
IHC-P	★★★★★ (3)	1/200. Perform heat mediated antigen retrieval before commencing with IHC staining protocol. <b>For unpurified use at 1/40</b> See <a href="#">IHC antigen retrieval protocols</a> .
ICC/IF	★★★★★ (4)	1/200. <b>For unpurified use at 1/40</b>

**Target****Function**

Plays a role in a process governing chromosomal dynamics during mitosis.

**Tissue specificity**

Ubiquitously expressed.

**Involvement in disease**

Note=A chromosomal aberration involving BRD4 is found in a rare, aggressive, and lethal carcinoma arising in midline organs of young people. Translocation t(15;19)(q14;p13) with NUT which produces a BRD4-NUT fusion protein.

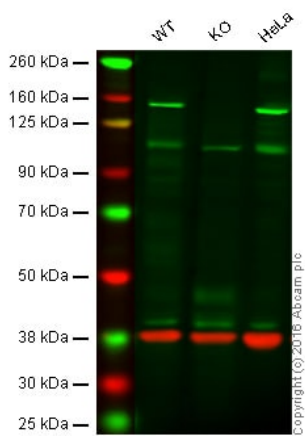
**Sequence similarities**

Contains 2 bromo domains.

**Cellular localization**

Nucleus.

**Images**



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

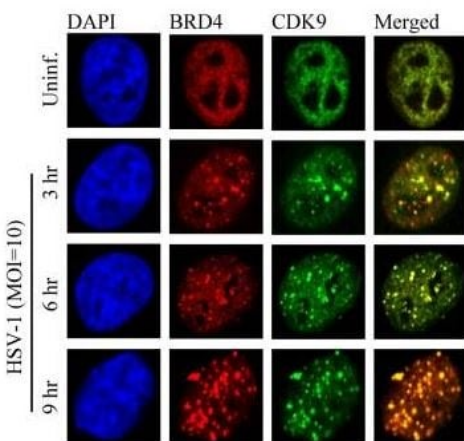
**Lane 1:** Wild-type HAP1 cell lysate (20 µg)

**Lane 2:** Brd4 knockout HAP1 cell lysate (20 µg)

**Lane 3:** HeLa cell lysate (20 µg)

**Lanes 1 - 4:** Merged signal (red and green). Green - ab128874 observed at 150 kDa. Red - loading control, ab8245, observed at 37 kDa.

ab128874 was shown to recognize Brd4 when Brd4 knockout samples were used, along with additional cross-reactive bands. Wild-type and Brd4 knockout samples were subjected to SDS-PAGE. ab128874 and ab8245 (loading control to GAPDH) were diluted at 1/1000 and 1/10 000 respectively and incubated overnight at 4°C. Blots were developed with Goat anti-Rabbit IgG H&L (IRDye® 800CW) preadsorbed (ab216773) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preadsorbed (ab216776) secondary antibodies at 1/10000 dilution for 1 hour at room temperature before imaging.



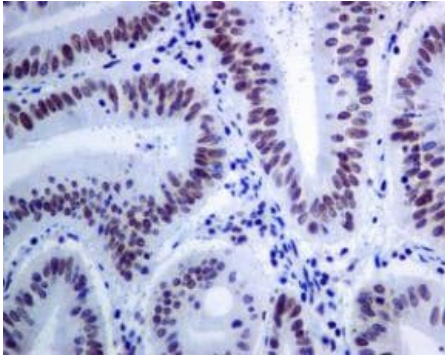
Immunocytochemistry/ Immunofluorescence - Anti-Brd4 antibody [EPR5150(2)] (ab128874)

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

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 permeabilized 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.

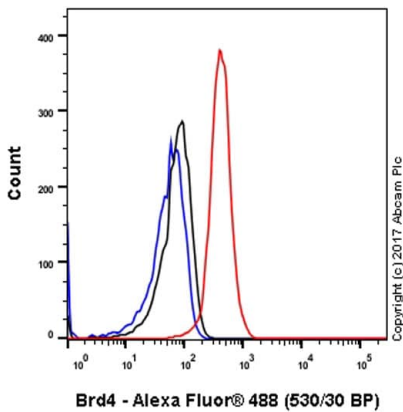
Reproduced under the Creative Commons Licence from Ren et al PLoS Pathog. 2016 Oct; 12(10): e1005950. Published online 2016 Oct 20. doi: 10.1371/journal.ppat.1005950



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

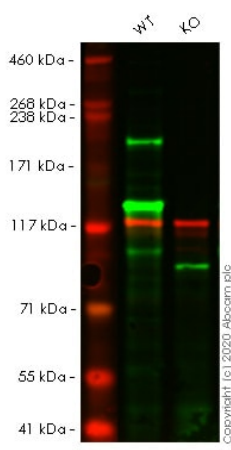
Unpurified ab128874 at 1/100 dilution staining Brd4 in human colon carcinoma tissue by Immunohistochemistry.

Perform heat mediated antigen retrieval before commencing with IHC staining protocol.



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

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 Fluor® 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).



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

**All lanes** : Anti-Brd4 antibody [EPR5150(2)] (ab128874) at 1/200 dilution

**Lane 1** : Wild-type HAP1 cell lysate

**Lane 2** : BRD4 knockout HAP1 cell lysate

Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

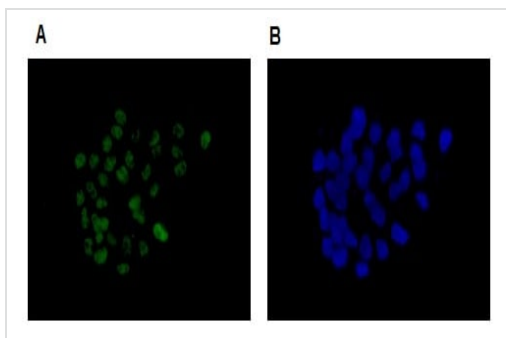
**Predicted band size:** 152 kDa

**Observed band size:** 220 kDa

**Lanes 1 - 2:** Merged signal (red and green). Green - ab128874

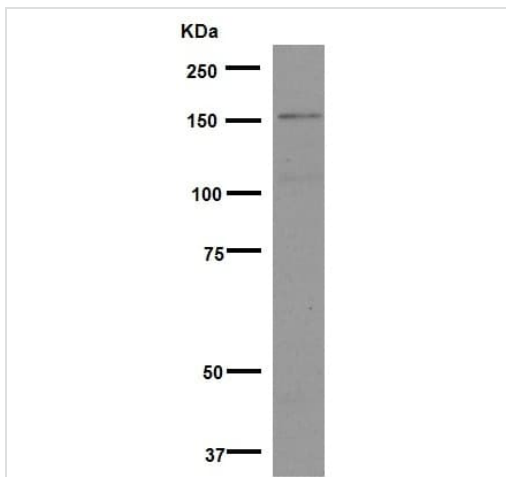
observed at 220 kDa. Red - loading control Mouse anti Vinculin observed at 125 kDa.

ab128874 was shown to react with Brd4 in wild-type cells in Western blot with loss of signal observed in BRD4 knockout sample. Wild-type and BRD4 knockout cell lysates were subjected to SDS-PAGE. Membranes were blocked in 3 % milk in TBS-T (0.1 % Tween<sup>®</sup>) before incubation with ab128874 and Mouse anti Vinculin overnight at 4 °C at a 1 in 200 dilution and a 1 in 20000 dilution respectively. Blots were incubated with Goat anti-Rabbit IgG H&L (IRDye<sup>®</sup> 800CW) preabsorbed (ab216773) and Goat anti-Mouse IgG H&L (IRDye<sup>®</sup> 680RD) preabsorbed (ab216776) secondary antibodies at 1 in 20000 dilution for 1 h at room temperature before imaging.



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

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



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

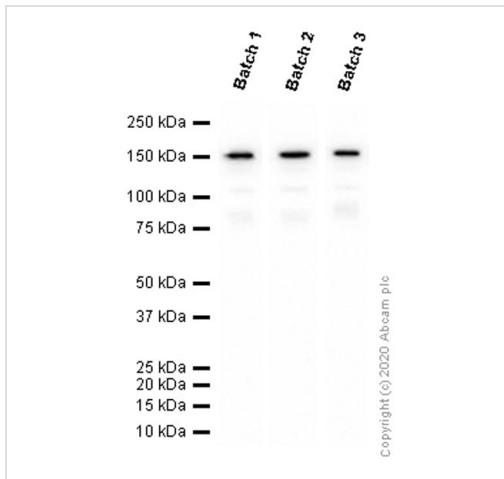
Anti-Brd4 antibody [EPR5150(2)] (ab128874) at 1/1000 dilution (Purified) + NIH/3T3 (Mouse embryo fibroblast cell line) cell lysate at 10 µg

#### Secondary

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

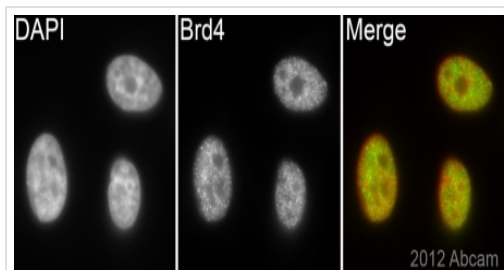
**Predicted band size:** 152 kDa

**Observed band size:** 152 kDa



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

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.

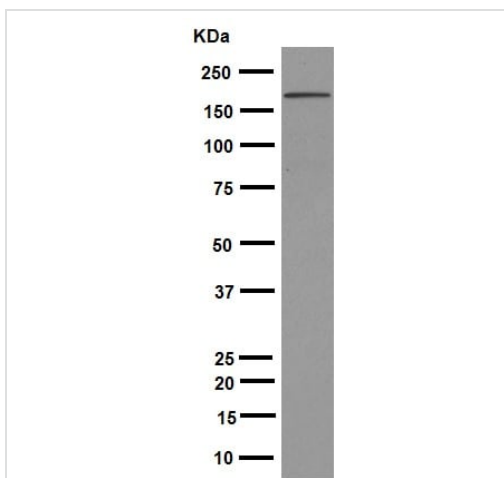


Immunocytochemistry/ Immunofluorescence - Anti-Brd4 antibody [EPR5150(2)] (ab128874)

Image courtesy of an abreview submitted by Dr. Kirk McManus, Univ. of Manitoba/Cancer Care MCB, Canada

Unpurified ab128874 (1/500) staining Brd4 in HeLa (Human epithelial cell line from cervix adenocarcinoma) cells (green). Cells were fixed in paraformaldehyde, permeabilized in 0.5% Triton X100/PBS and counterstained with DAPI in order to highlight the nucleus (red).

For further experimental details please refer to Abreview.



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

Anti-Brd4 antibody [EPR5150(2)] (ab128874) at 1/1000 dilution (purified) + HeLa (Human epithelial cell line from cervix adenocarcinoma) cell lysate at 10 µg

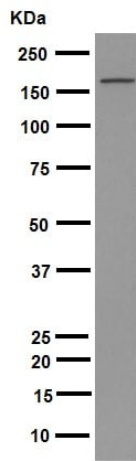
### Secondary

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

**Predicted band size:** 152 kDa

**Observed band size:** 152 kDa

Blocking and Diluting buffer and concentration: 5% NFD/MTBST



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

Anti-Brd4 antibody [EPR5150(2)] (ab128874) at 1/200 dilution (unpurified) + HeLa (Human epithelial cell line from cervix adenocarcinoma) cell lysate at 10 µg/ml

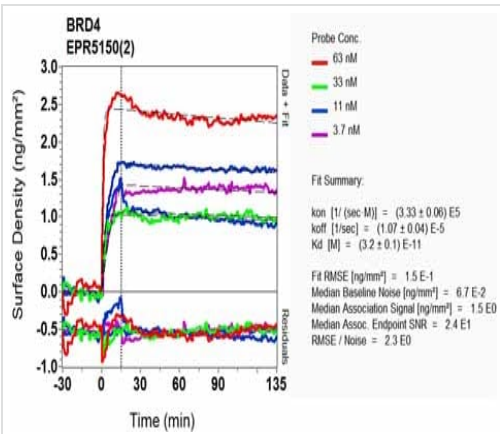
### Secondary

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

**Predicted band size:** 152 kDa

**Observed band size:** 152 kDa

Blocking and Diluting buffer and concentration: 5% NFDM/TBST



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

Equilibrium dissociation constant ( $K_D$ )

Learn more about  $K_D$

[Click here to learn more about  \$K\_D\$](#)

### Why choose a recombinant antibody?



**Research with confidence**  
Consistent and reproducible results



**Long-term and scalable supply**  
Recombinant technology



**Success from the first experiment**  
Confirmed specificity



**Ethical standards compliant**  
Animal-free production

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

**Please note:** All products are "FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES"

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