


## Product datasheet

### Anti-Brd4 antibody [EPR5150(2)] $\alpha$ b128874

KO VALIDATED Recombinant RabMAb

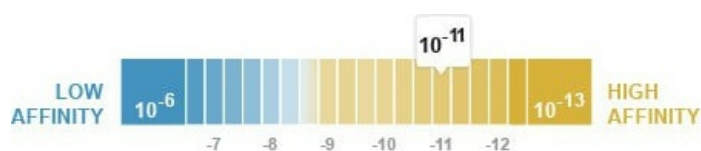
★★★★☆ 17 Abreviews 139 References 13 Images

#### Overview

Product name	Anti-Brd4 antibody [EPR5150(2)]
Description	Rabbit monoclonal [EPR5150(2)] to Brd4
Host species	Rabbit
Tested applications	<b>Suitable for:</b> Flow Cyt (Intra), WB, IHC-P, ICC/IF
Species reactivity	<b>Reacts with:</b> Mouse, Human <b>Predicted to work with:</b> Rat 
Immunogen	Synthetic peptide within Human Brd4 aa 150-250. The exact sequence is proprietary. Database link: <a href="#">O60885</a>
Positive control	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.
General notes	This product is a recombinant monoclonal antibody, which offers several advantages including: <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> For more information <a href="#">see here</a> . 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> .

#### Properties

Form	Liquid
Storage instructions	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 ( $K_D$ )	$K_D = 3.20 \times 10^{-11}$ M



## [Learn more about K<sub>D</sub>](#)

<b>Storage buffer</b>	pH: 7.20 Preservative: 0.01% Sodium azide Constituents: PBS, 40% Glycerol, 0.05% BSA
<b>Purity</b>	Protein A purified
<b>Clonality</b>	Monoclonal
<b>Clone number</b>	EPR5150(2)
<b>Isotype</b>	IgG

## Applications

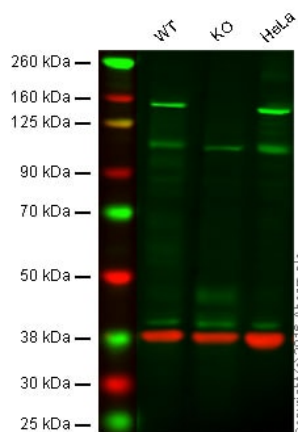
**The Abpromise guarantee** Our **Abpromise guarantee** covers the use of ab128874 in the following tested applications. The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

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

<b>Function</b>	Plays a role in a process governing chromosomal dynamics during mitosis.
<b>Tissue specificity</b>	Ubiquitously expressed.
<b>Involvement in disease</b>	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.
<b>Sequence similarities</b>	Contains 2 bromo domains.
<b>Cellular localization</b>	Nucleus.

## Images



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

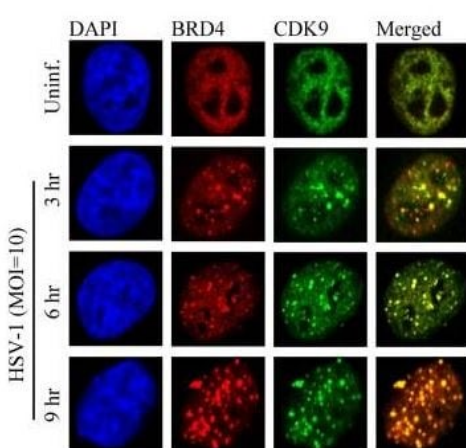
**Lane 1:** Wild-type HAP1 cell lysate (20 µg)

**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.



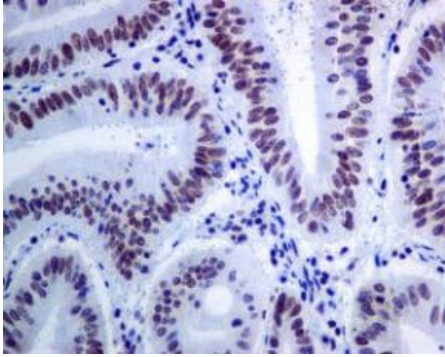
Immunocytochemistry/ Immunofluorescence - Anti-Brd4 antibody [EPR5150(2)] (ab128874)

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.

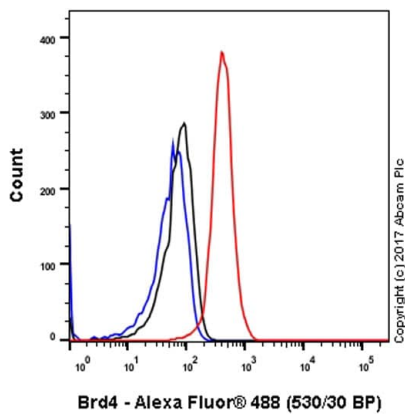
Reproduced under the Creative Commons Licence from Ren et al **PLoS Pathog.** 2016 Oct; 12(10): e1005950. Published online 2016 Oct 20. doi: [10.1371/journal.ppat.1005950](https://doi.org/10.1371/journal.ppat.1005950)



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

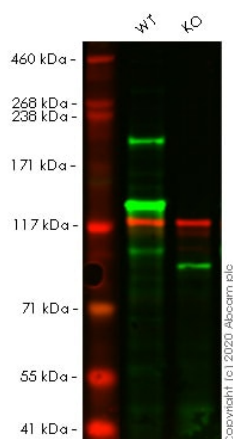
Unpurified ab128874 at 1/100 dilution staining Brd4 in human colon carcinoma tissue by Immunohistochemistry.

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.

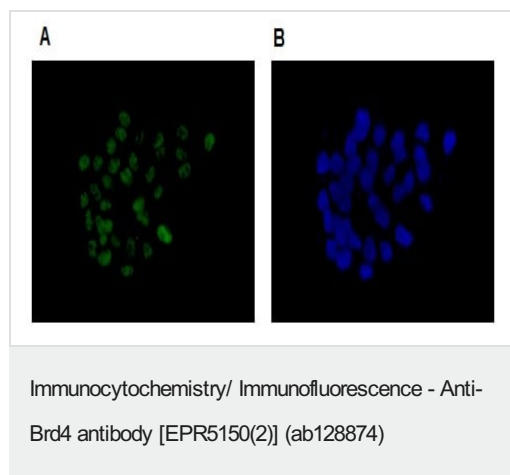
Performed under reducing conditions.

**Predicted band size:** 152 kDa

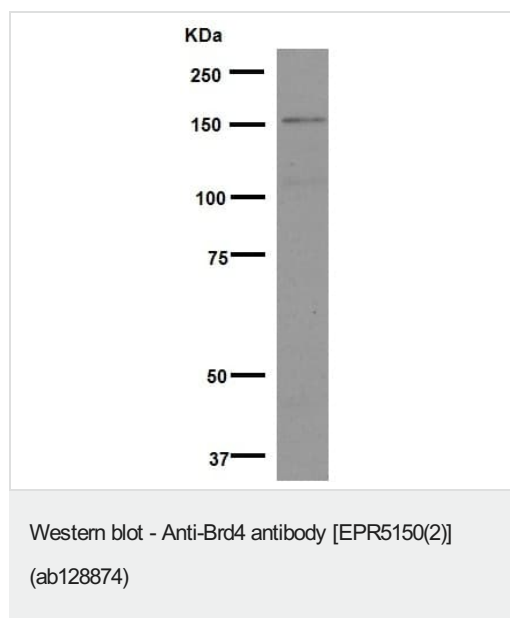
**Observed band size:** 220 kDa

**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®) 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® 800CW) preabsorbed ([ab216773](#)) and Goat anti-Mouse IgG H&L (IRDye® 680RD) preabsorbed ([ab216776](#)) secondary antibodies at 1 in 20000 dilution for 1 h at room temperature before imaging.



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).



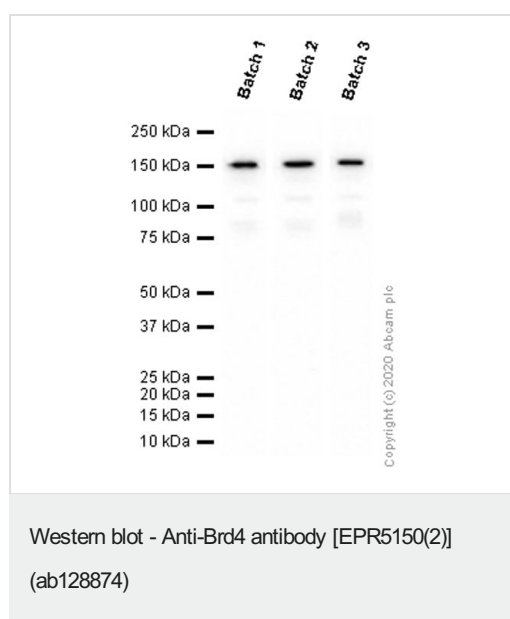
Anti-Brd4 antibody [EPR5150(2)] (ab128874) at 1/1000 dilution (Purified) + NIH/3T3 (Mouse embryo fibroblast cell line) cell lysate at 10 µg

#### Secondary

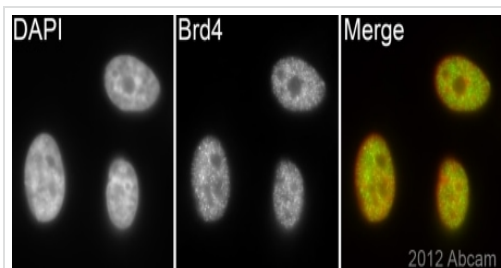
Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated at 1/1000 dilution

**Predicted band size:** 152 kDa

**Observed band size:** 152 kDa



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.

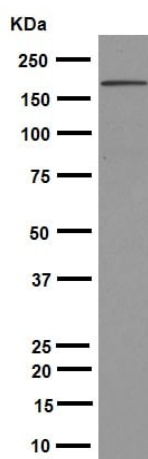


Immunocytochemistry/ Immunofluorescence - Anti-Brd4 antibody [EPR5150(2)] (ab128874)

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

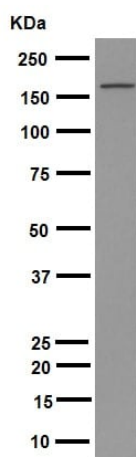
#### Secondary

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% NFDM/TBST



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

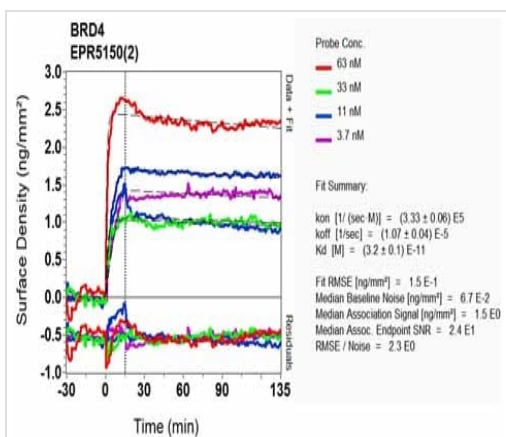
#### Secondary

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



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

Equilibrium disassociation 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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