abcam

Product datasheet

Anti-Brd4 antibody [EPR5150(2)] ab128874

KO 評価済 リコンビナント RabMAb

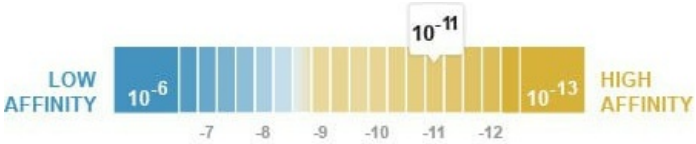
17 Abreviews 139 References 画像数 13

製品の概要

製品名	Anti-Brd4 antibody [EPR5150(2)]
製品の詳細	Rabbit monoclonal [EPR5150(2)] to Brd4
由来種	Rabbit
アプリケーション	**適用あり:** Flow Cyt (Intra), WB, IHC-P, ICC/IF
種交差性	**交差種:** Mouse, Human **交差が予測される動物種:** Rat
免疫原	Synthetic peptide within Human Brd4 aa 150-250. The exact sequence is proprietary. Database link: **O60885**
ポジティブ・コントロール	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.
特記事項	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 production For more information **see here**. Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to **RabMAb® patents**.

製品の特性

製品の状態	Liquid
保存方法	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.
解離定数（K_D 値）	K_D = 3.20 x 10^{-11} M



Learn more about K_D

バッファー	pH: 7.20 Preservative: 0.01% Sodium azide Constituents: PBS, 40% Glycerol, 0.05% BSA
精製度	Protein A purified
ポリ/モノ	モノクローナル
クローン名	EPR5150(2)
アイソタイプ	IgG

アプリケーション

The Abpromise guarantee Abpromise保証は、次のテスト済みアプリケーションにおけるab128874の使用に適用されます

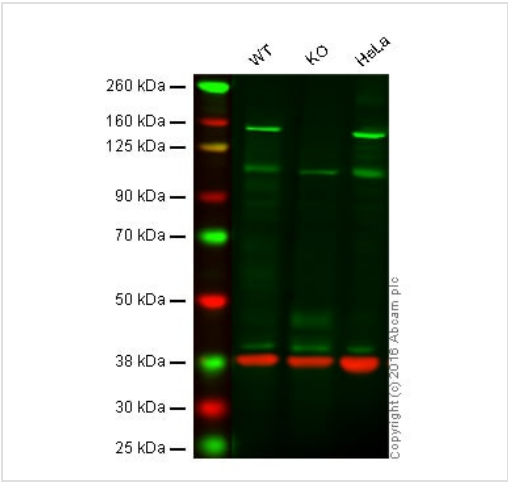
アプリケーションノートには、推奨の開始希釈率がありますが、適切な希釈率につきましてはご検討ください。

アプリケーション	Abreviews	特記事項
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. **For unpurified use at 1/40** See **IHC antigen retrieval protocols**.
ICC/IF	★★★★★ (4)	1/200. **For unpurified use at 1/40**

ターゲット情報

機能	Plays a role in a process governing chromosomal dynamics during mitosis.
組織特異性	Ubiquitously expressed.
関連疾患	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.
配列類似性	Contains 2 bromo domains.
細胞内局在	Nucleus.

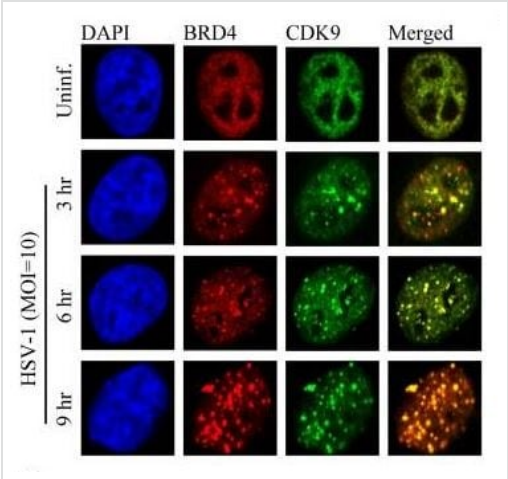
画像



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.

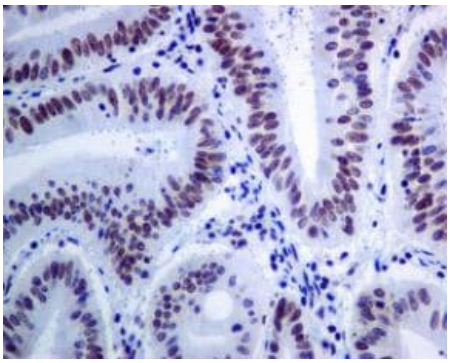


Immunocytochemistry/ Immunofluorescence - Anti-Brd4 antibody [EPR5150(2)] (ab128874)

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 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).

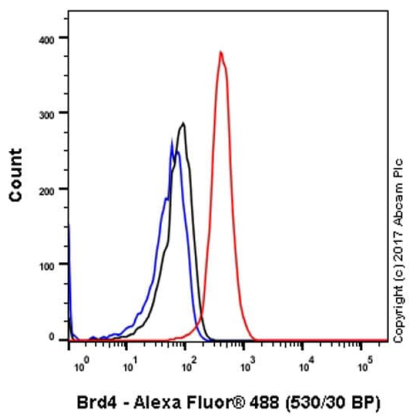
From Figure 5b of Ren et al.



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

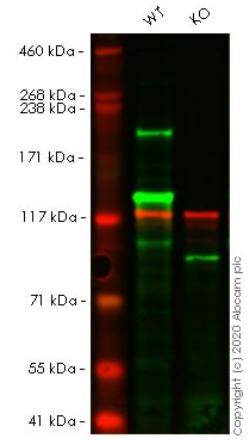
Unpurified ab128874 at 1/100 dilution staining Brd4 in human colon carcinoma tissue by Immunohistochemistry.

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

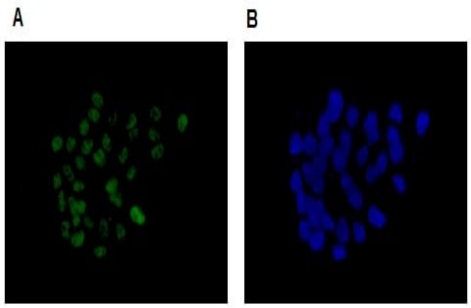
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 152 kDa
Observed band size: 220 kDa

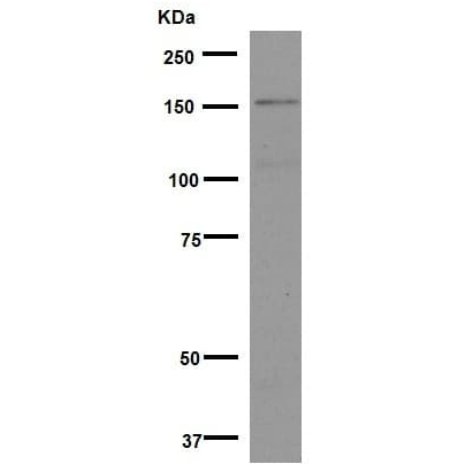
Lanes 1 - 2: Merged signal (red and green). Green - ab128874 observed at 220 kDa. Red - loading control Mouse anti Vinculin observed at 125 kDa.

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 - 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® 488-conjugated goat anti-rabbit IgG (1/200) was used as the secondary antibody. DAPI (blue) was used as the nuclear counterstain(Panel B).

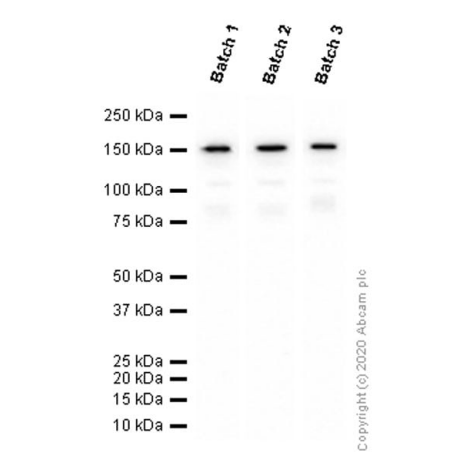


Western blot - Anti-Brd4 antibody [EPR5150(2)] (ab128874)

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

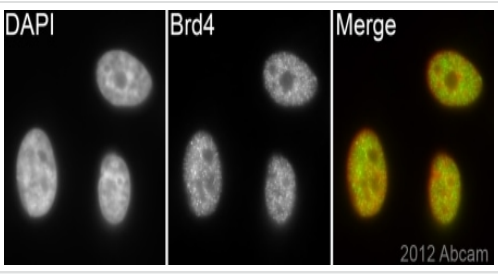
Secondary
Goat Anti-Rabbit IgG, (H+L), Peroxidase conjugated at 1/1000 dilution

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.

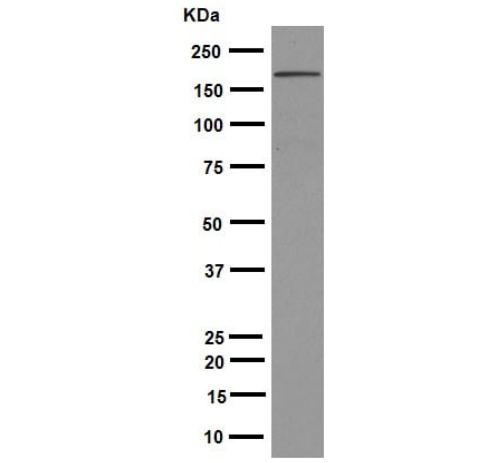


Immunocytochemistry/ Immunofluorescence - Anti-Brd4 antibody [EPR5150(2)] (ab128874)

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



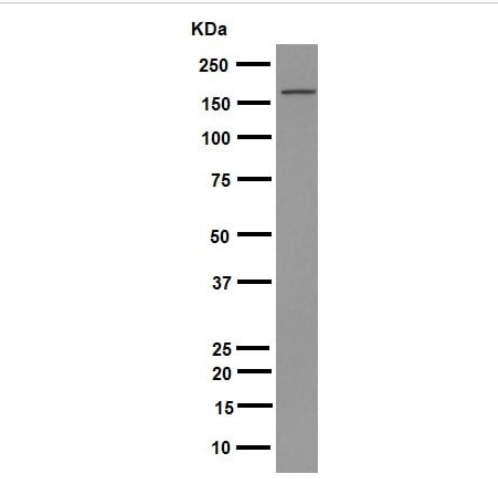
Western blot - Anti-Brd4 antibody [EPR5150(2)] (ab128874)

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



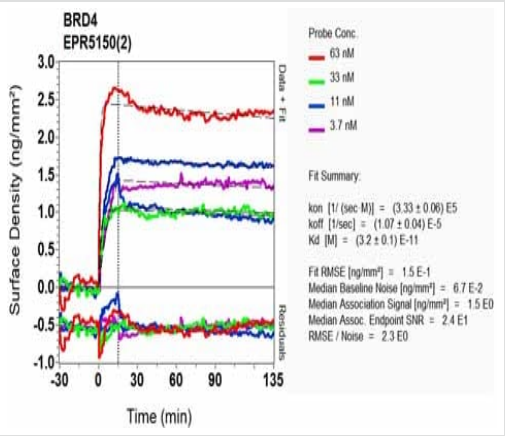
Western blot - Anti-Brd4 antibody [EPR5150(2)] (ab128874)

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

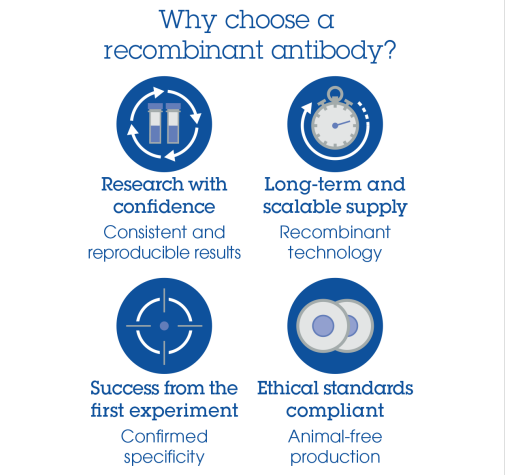


OI-RD Scanning - Anti-Brd4 antibody [EPR5150(2)] (ab128874)

Equilibrium disassociation constant (K_D)

Learn more about K_D

Click here to learn more about K_D



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

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