abcam

Product datasheet

Anti-Brd4 antibody ab75898





Overview

Product name Anti-Brd4 antibody

Description Rabbit polyclonal to Brd4

Host species Rabbit

Tested applications Suitable for: ICC/IF, WB

Species reactivity Reacts with: Mouse, Rat, Human

Predicted to work with: Horse, Dog

Immunogen Synthetic peptide corresponding to Human Brd4 aa 1100-1200 conjugated to keyhole limpet

haemocyanin.

(Peptide available as ab85811)

Positive control WB: HAP1 and HeLa cell lysates; Mouse and rat liver tissue lysates. ICC/IF: MCF7 cells.

General notes "Note: Batches of this product that have a concentration < 1mg/ml may have BSA added as a

stabilising agent. If you would like information about the formulation of a specific lot, please

contact our scientific support team who will be happy to help."

The Life Science industry has been in the grips of a reproducibility crisis for a number of years. Abcam is leading the way in addressing this with our range of recombinant monoclonal antibodies and knockout edited cell lines for gold-standard validation. Please check that this product meets

your needs before purchasing.

If you have any questions, special requirements or concerns, please send us an inquiry and/or contact our Support team ahead of purchase. Recommended alternatives for this product can be

found below, along with publications, customer reviews and Q&As

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 or -

80°C. Avoid freeze / thaw cycle.

Storage buffer pH: 7.40

Preservative: 0.02% Sodium azide

Constituent: PBS

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Purity Immunogen affinity purified

Clonality Polyclonal

Isotype IgG

Applications

The Abpromise guarantee Our <u>Abpromise guarantee</u> covers the use of ab75898 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
ICC/IF	*** <u>*</u>	Use a concentration of 5 µg/ml.
WB	★ ★ ★ ★ ★ (1)	Use a concentration of 1 µg/ml. Detects a band of approximately 152 kDa (predicted molecular weight: 152 kDa).

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

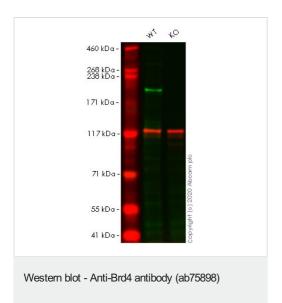
carcinoma arising in midline organs of young people. Translocation t(15;19)(q14;p13) with NUT

which produces a BRD4-NUT fusion protein.

Sequence similarities Contains 2 bromo domains.

Cellular localization Nucleus.

Images



All lanes: Anti-Brd4 antibody (ab75898) at 1 µg/ml

Lane 1: Wild-type HAP1 cell lysate

Lane 2: BRD4 knockout HAP1 cell lysate

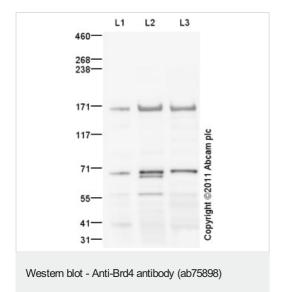
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

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

Lanes 1 - 2: Merged signal (red and green). Green - ab75898 observed at 220 kDa. Red - loading control Mouse anti Vinculin observed at 125 kDa.

ab75898 was shown to react with Brd4 in HAP1 wild-type cells in Western blot with loss of signal observed in BRD4 knockout sample. Wild-type HAP1 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 ab75898 and Mouse anti Vinculin overnight at 4 °C at 1 μ g/ml and a 1 in 20000 dilution respectively. Blots were incubated with Goat anti-Rabbit lgG H&L (IRDye® 800CW) preabsorbed (ab216773) and Goat anti-Mouse lgG H&L (IRDye® 680RD) preabsorbed (ab216776) secondary antibodies at 1 in 20000 dilution for 1 h at room temperature before imaging.



All lanes: Anti-Brd4 antibody (ab75898) at 1 µg/ml

Lane 1: Liver (Mouse) Tissue Lysate

Lane 2: Liver (Rat) Tissue Lysate

Lane 3: Human liver tissue lysate - total protein (ab29889)

Lysates/proteins at 10 µg/ml per lane.

Secondary

All lanes : Goat polyclonal to Rabbit lgG - H&L - Pre-Adsorbed (HRP)

Developed using the ECL technique.

Performed under reducing conditions.

Predicted band size: 152 kDa **Observed band size:** 171 kDa

Additional bands at: 70 kDa. We are unsure as to the identity of

these extra bands.

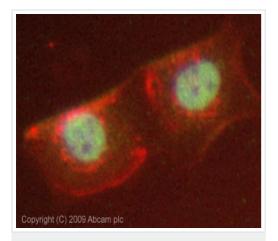
Exposure time: 150 seconds

DAPI BRD4 Merge

Immunocytochemistry/ Immunofluorescence - Anti-Brd4 antibody (ab75898)

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

ab75898 (1/500) staining Brd4 in HeLa cells (green). Cells were fixed in paraformaldehyde, permeabilized with 0.5% Triton X-100/PBS and counterstained with DAPI in order to highlight the nucleus (red). For further experimental details please refer to Abreview.



Immunocytochemistry/ Immunofluorescence - Anti-Brd4 antibody (ab75898)

ICC/IF image of ab75898 stained MCF7 cells. The cells were 4% PFA fixed (10 min) and then incubated in 1%BSA / 10% normal goat serum / 0.3M glycine in 0.1% PBS-Tween for 1h to permeabilise the cells and block non-specific protein-protein interactions. The cells were then incubated with the antibody (ab75898, 5µg/ml) overnight at +4°C. The secondary antibody (green) was Alexa Fluor® 488 goat anti-rabbit IgG (H+L) used at a 1/1000 dilution for 1h. Alexa Fluor® 594 WGA was used to label plasma membranes (red) at a 1/200 dilution for 1h. DAPI was used to stain the cell nuclei (blue) at a concentration of 1.43µM. This antibody also gave a positive result in 4% PFA fixed (10 min) HeLa, Hek293 and HepG2 cells at 5µg/ml.

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

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