abcam

Product datasheet

Anti-CENPB antibody ab25734



★★★★★ 6 Abreviews 44 References 5 Images

Overview

Product name Anti-CENPB antibody

Description Rabbit polyclonal to CENPB

Host species Rabbit

Tested applications Suitable for: WB, ICC/IF, ICC

Species reactivity Reacts with: Human

Predicted to work with: Mouse, Rat, Sheep, Hamster

Immunogen Synthetic peptide conjugated to KLH derived from within residues 550 to the C-terminus of

Human CENPB. Read Abcam's proprietary immunogen policy (Peptide available as ab28019.)

Positive control ICC: HeLa cells. WB: A431, Jurkat, Daudi and HeLa cell lysates.

General notes

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

Batches of this product that have a concentration < 1mg/ml may have BSA added as a stabilising

 $agent. \ \ \ \ \ formulation\ of\ a\ specific\ lot,\ please\ contact\ our$

scientific support team who will be happy to help.

Purity Immunogen affinity purified

1

Clonality Polyclonal

Isotype IgG

Applications

The Abpromise guarantee Our Abpromise guarantee covers the use of ab25734 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
WB	****(1)	Use a concentration of 1 - 5 µg/ml. Detects a band of approximately 65 kDa (predicted molecular weight: 65 kDa).
ICC/IF	★★★★★ (5)	1/200 - 1/1000.
ICC		Use a concentration of 1 µg/ml.

Target

Function Interacts with centromeric heterochromatin in chromosomes and binds to a specific subset of

alphoid satellite DNA, called the CENP-B box. May organize arrays of centromere satellite DNA into a higher order structure which then directs centromere formation and kinetochore assembly in

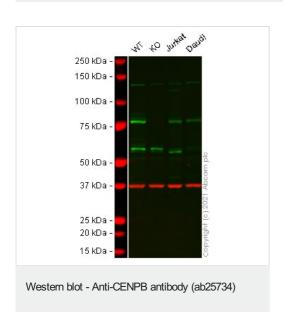
mammalian chromosomes.

Sequence similaritiesContains 1 HTH CENPB-type DNA-binding domain.

Contains 1 HTH psq-type DNA-binding domain.

Cellular localization Nucleus. Chromosome > centromere.

Images



All lanes: Anti-CENPB antibody (ab25734) at 1 μg/ml

Lane 1 : Wild-type A-431 (Human epidermoid carcinoma cell line)

whole cell lysate

Lane 2: CENPB knockout A-431 (Human epidermoid carcinoma

cell line) whole cell lysate

Lane 3: Jurkat (Human T cell leukemia cell line from peripheral

blood) whole cell lysate

Lane 4: Daudi (Human Burkitt's lymphoma cell line) whole cell

lysate

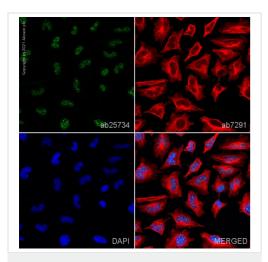
Lysates/proteins at 20 µg per lane.

Performed under reducing conditions.

Predicted band size: 65 kDa **Observed band size:** 77 kDa

Lanes 1 - 4: Merged signal (red and green). Green - ab25734 observed at 77 kDa. Red - loading control <u>ab8245</u> (Mouse anti-GAPDH antibody [6C5]) observed at 37 kDa.

ab25734 was shown to react with CENPB in wild-type A431 cells in Western blot with loss of signal observed in CENPB knockout sample.Wild-type A431 and CENPB knockout cell lysates were subjected to SDS-PAGE. Membranes were blocked in 3 % milk in TBS-T (0.1 % Tween®) before incubation with ab25734 and ab8245 (Mouse anti-GAPDH antibody [6C5]) overnight at 4°C at 1 µg/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.



Immunocytochemistry/ Immunofluorescence - Anti-CENPB antibody (ab25734)

ab25734 staining CENPB in HeLa cells. The cells were fixed with 4% paraformaldehyde (10 min), permeabilized with 0.1% PBS-Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1%PBS-Tween for 1h. The cells were then incubated overnight at 4°C with ab25734 at 1 µg/ml and ab7291, Mouse monoclonal [DM1A] to alpha Tubulin - Loading Control. Cells were then incubated with ab150120, Goat polyclonal Secondary Antibody to Mouse IgG - H&L (Alexa Fluor[®] 594), preadsorbed at 1/1000 dilution (shown in green) and ab150081, Goat polyclonal Secondary Antibody to Rabbit IgG - H&L (Alexa Fluor[®] 488), pre-adsorbed at 1/1000 dilution (shown in pseudocolour red). Nuclear DNA was labelled with DAPI (shown in blue).

Also suitable in cells fixed with 100% methanol (5 min).

Image was acquired with a high-content analyser (Operetta CLS, Perkin Elmer) and a maximum intensity projection of confocal sections is shown.

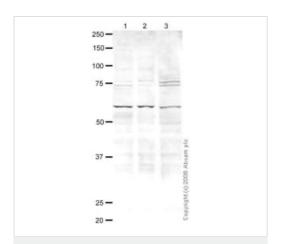


Immunocytochemistry/ Immunofluorescence - Anti-CENPB antibody (ab25734)

Image courtesy of an AbReview by Weiguo Zhang

4% paraformaldehyde-fixed, 0.2% Triton X-100 permeabilized HeLa (human epithelial cell line from cervix adenocarcinoma) cells stained for CENPB (red) using ab25734 at 1/200 dilution in ICC/IF.

The nuclear counterstain is DAPI (blue). Alpha-Tubulin is stained with an mouse monoclonal anti-alpha Tubulin antibody (green).



Western blot - Anti-CENPB antibody (ab25734)

All lanes: Anti-CENPB antibody (ab25734) at 1 µg/ml

Lane 1 : HeLa (Human epithelial carcinoma cell line) Whole Cell

Lysate

Lane 2 : Jurkat whole cell lysate (ab7899)

Lane 3: A-431 whole cell lysate (ab7909)

Lysates/proteins at 20 µg per lane.

Secondary

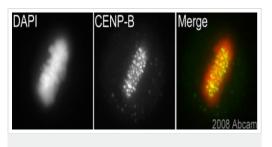
All lanes : Goat polyclonal to Rabbit lgG (Alexa Fluor® 680) at 1/10000 dilution

Developed using the ECL technique.

Predicted band size: 65 kDa **Observed band size:** 65 kDa

Additional bands at: 74 kDa (possible cross reactivity), 75 kDa

(possible cross reactivity)



ab25734 (1/1000) staining CENPB in HeLa cells (green). Cells were fixed with methanol and counterstained with DAPI in order to highlight the nucleus (red). Please refer to abreview for further experimental details.

Immunocytochemistry - Anti-CENPB antibody (ab25734)

This image is courtesy of an Abreview submitted by Dr Kirk McManus

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