# abcam

# Product datasheet

# Anti-DCAMKL1 antibody ab31704



\*\*\* 14 Abreviews 106 References 7 Images

#### Overview

Product name Anti-DCAMKL1 antibody

**Description** Rabbit polyclonal to DCAMKL1

Host species Rabbit

**Specificity** This antibody recognizes 2 different human isoforms (AL: 82.2 KDa and BL: 47.6 KDa)

Tested applications Suitable for: ICC, WB, IP

Species reactivity Reacts with: Mouse, Rat, Human

**Immunogen** Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.

Positive control WB: HAP1 and NIH-3T3 cell lysates. Human, mouse, and rat brain tissue 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. If you would like information about the formulation of a specific lot, please contact our

scientific support team who will be happy to help

**Purity** Immunogen affinity purified

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**Clonality** Polyclonal

**Isotype** IgG

#### **Applications**

# The Abpromise guarantee

Our **Abpromise guarantee** covers the use of ab31704 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	*** <u>*</u>	Use a concentration of 5 µg/ml.
WB	★★★★☆ (4)	Use a concentration of 0.5 µg/ml. Detects a band of approximately 47, 82 kDa (predicted molecular weight: 47, 82 kDa).
IP		Use at an assay dependent concentration.

# **Target**

**Function** Probable kinase that may be involved in a calcium-signaling pathway controlling neuronal

migration in the developing brain. May also participate in functions of the mature nervous system.

Tissue specificity In fetal tissues, highly expressed in brain, detectable in lung and liver, but not in kidney. In adult

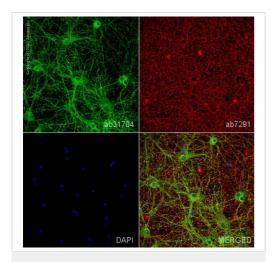
tissues, expressed ubiquitously in the brain, detectable in the heart, liver, spleen, thymus, prostate, testis, ovary, small intestine and colon. The type A isoforms seem to be expressed predominantly

in fetal brain whereas type B isoforms are expressed abundantly in both fetal and adult brain.

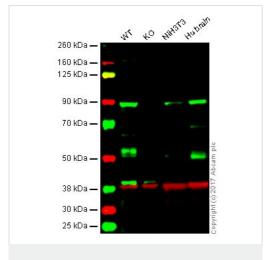
**Sequence similarities**Belongs to the protein kinase superfamily. CAMK Ser/Thr protein kinase family. CaMK subfamily.

Contains 2 doublecortin domains.
Contains 1 protein kinase domain.

#### **Images**



Immunocytochemistry - Anti-DCAMKL1 antibody (ab31704)



Western blot - Anti-DCAMKL1 antibody (ab31704)

ab31704 staining DCAMKL1 in primary hippocampal rat neurons/glia, (obtained from Neuromics, cat. no. PC35101), DIV14. The cells were fixed with 4% paraformaldehyde (10 min), permeabilized with 0.1% PBS-Tween 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 ab31704 at 5 µg/ml and ab7291, Mouse monoclonal [DM1A] to alpha Tubulin - Loading Control. Cells were then incubated with ab150081, Goat polyclonal Secondary Antibody to Rabbit lgG - H&L (Alexa Fluor® 488), pre-adsorbed at 1/1000 dilution (shown in green) and ab150120, Goat polyclonal Secondary Antibody to Mouse lgG - H&L (Alexa Fluor® 594), pre-adsorbed at 1/1000 dilution (shown in pseudocolour red). Nuclear DNA was labelled with DAPI (shown in blue).

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

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

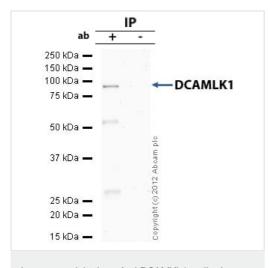
Lane 2: DCAMKL1 knockout HAP1 whole cell lysate (20 µg)

Lane 3: NIH3T3 whole cell lysate (20 µg)

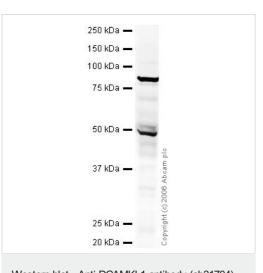
Lane 4: Human brain whole tissue lysate (20 µg)

**Lanes 1 - 4:** Merged signal (red and green). Green - ab31704 observed at 90 kDa. Red - loading control, <u>ab8245</u>, observed at 37 kDa.

ab31704 was shown to specifically react with DCAMKL1 in wild-type HAP1 cells along with additional cross reactive bands. No bands were observed when DCAMKL1 knockout cells were examined. Wild-type and DCAMKL1 knockout samples were subjected to SDS-PAGE. ab31704 and <a href="mailto:ab8245">ab8245</a> (Mouse anti-GAPDH loading control) were incubated overnight at 4°C at 1 µg/ml and 1/10,000 dilution respectively. Blots were developed 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 1/10,000 dilution for 1 hour at room temperature before imaging.



Immunoprecipitation - Anti-DCAMKL1 antibody (ab31704)



Western blot - Anti-DCAMKL1 antibody (ab31704)

DCAMKL1 was immunoprecipitated using 0.5mg Mouse Brain whole tissue lysate, 5µg of Rabbit polyclonal to DCAMKL1 and 50µl of protein G magnetic beads (+). No antibody was added to the control (-).

The antibody was incubated under agitation with Protein G beads for 10min, Mouse Brain whole tissue lysate lysate diluted in RIPA buffer was added to each sample and incubated for a further 10min under agitation.

Proteins were eluted by addition of  $40\mu I$  SDS loading buffer and incubated for 10min at  $70^{o}C$ ;  $10\mu I$  of each sample was separated on a SDS PAGE gel, transferred to a nitrocellulose membrane, blocked with 5% BSA and probed with ab31704.

Secondary: Mouse monoclonal [SB62a] Secondary Antibody to Rabbit lgG light chain (HRP) (ab99697).

Band: 82kDa: DCAMKL1; non specific - 52 and 27kDa: We are unsure as to the identity of this extra band.

Anti-DCAMKL1 antibody (ab31704) at 1  $\mu$ g/ml + Mouse Brain Whole Tissue Lysate at 20  $\mu$ g

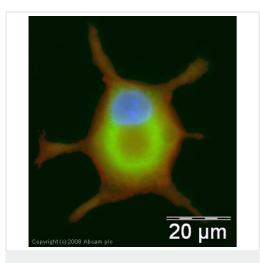
## Secondary

IR Dye 680 Conjugated Goat Anti-Rabbit lgG (H+L) at 1/15000 dilution

Performed under reducing conditions.

Predicted band size: 47, 82 kDa Observed band size: 47,82 kDa

The 82 kDa and 47 kDa bands correspond to the AL and BL isoforms respectively. This antibody should not detect the AS and BS isoforms of DCAMLK1.



Immunocytochemistry - Anti-DCAMKL1 antibody (ab31704)

ICC/IF image of ab31704 stained PC12 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 (ab31704, 1µg/ml) overnight at +4°C. The secondary antibody (green) was Alexa Fluor® 488 goat anti-rabbit lgG (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).



Western blot - Anti-DCAMKL1 antibody (ab31704)

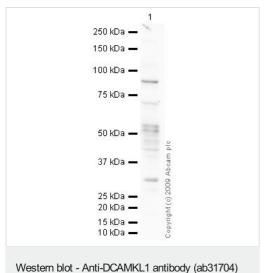
Anti-DCAMKL1 antibody (ab31704) at 1  $\mu$ g/ml + Brain (Rat) Tissue Lysate - normal tissue at 10  $\mu$ g

#### **Secondary**

IRDye 680 Conjugated Goat Anti-Rabbit IgG (H+L) at 1/10000 dilution

Performed under reducing conditions.

**Predicted band size:** 47, 82 kDa **Observed band size:** 47,82 kDa



vestern blot - Anti-DCAlvirL1 antibody (ab31704)

Anti-DCAMKL1 antibody (ab31704) at 1 μg/ml + Human brain tissue lysate - total protein (**ab29466**) at 10 μg

#### **Secondary**

Goat polyclonal to Rabbit lgG - H&L - Pre-Adsorbed (HRP) at 1/3000 dilution

Performed under reducing conditions.

**Predicted band size:** 47, 82 kDa **Observed band size:** 47,82 kDa

**Additional bands at:** 30 kDa, 52 kDa (possible post-translational modification), 54 kDa (possible post-translational modification). We

are unsure as to the identity of these extra bands.

Exposure time: 5 minutes

The 82 kDa and 47 kDa bands correspond to the AL and BL isoforms respectively. DCAMLK1 has a number of potential phosphorylation sites which may explain the higher migrating bands at 52 and 54 kDa.

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

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