abcam

Product datasheet

Anti-Hippocalcin antibody ab24560

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Overview

Product name Anti-Hippocalcin antibody

Description Rabbit polyclonal to Hippocalcin

Host species Rabbit

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

Species reactivity Reacts with: Mouse, Rat, Human, Zebrafish

Predicted to work with: Chicken, Cow

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

Positive control ICC/IF: primary hippocampal rat neurons/glia, DIV14. cells WB: Rat Brain, Mouse Brain. IP:

Mouse Brain.

General notesThe 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

Clonality Polyclonal

1

Isotype IgG

Applications

The Abpromise guarantee Our <u>Abpromise guarantee</u> covers the use of ab24560 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		Use a concentration of 1 µg/ml. Detects a band of approximately 23 kDa (predicted molecular weight: 23 kDa).
ICC/IF		Use a concentration of 5 µg/ml.
IP		Use at an assay dependent concentration.

Target

Function May be involved in the calcium-dependent regulation of rhodopsin phosphorylation. Binds two

calcium ions.

Tissue specificity Brain specific.

Sequence similarities Belongs to the recoverin family.

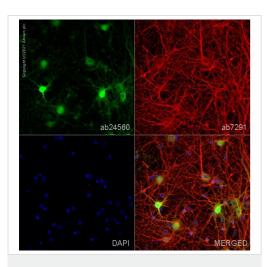
Contains 4 EF-hand domains.

Post-translational

modifications

Myristoylation facilitates interaction with membranes.

Images

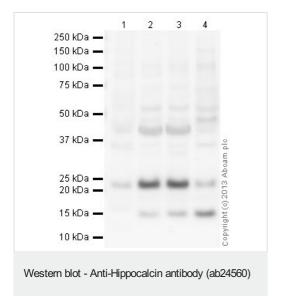


Immunocytochemistry/ Immunofluorescence - Anti-Hippocalcin antibody (ab24560)

ab24560 staining Hippocalcin in primary hippocampal rat neurons/glia, (obtained from Neuromics, cat. no. PC35101), DIV14. 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 ab24560 at 5µg/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 ab150080, Goat polyclonal Secondary Antibody to Rabbit lgG - H&L (Alexa Fluor® 594) 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.



All lanes: Anti-Hippocalcin antibody (ab24560) at 1 µg/ml

Lane 1: Human brain tissue lysate - total protein (ab29466)

Lane 2: Brain (Rat) Tissue Lysate

Lane 3: Brain (Mouse) Tissue Lysate

Lane 4: Mouse brain tissue lysate - total protein (0 days) (ab7188)

Lysates/proteins at 20 µg per lane.

Secondary

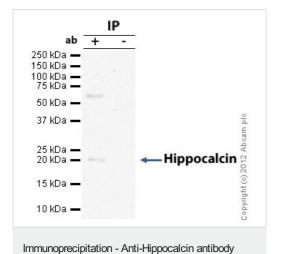
All lanes : Goat Anti-Rabbit IgG H&L (HRP) (<u>ab97051</u>) at 1/10000 dilution

Developed using the ECL technique.

Performed under reducing conditions.

Predicted band size: 23 kDa **Observed band size:** 23 kDa

Additional bands at: 14 kDa (possible cleavage fragment), 42 kDa, 52 kDa. We are unsure as to the identity of these extra bands.



(ab24560)

Exposure time: 4 minutes

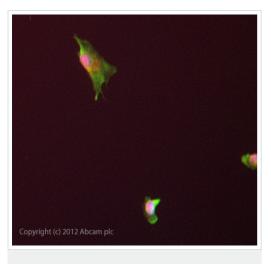
Hippocalcin was immunoprecipitated using 0.5mg Mouse Brain whole tissue lysate, 5µg of Rabbit polyclonal to Hippocalcin and 50µl of protein G magnetic beads (+). No antibody was added to the control (-).

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 40μ I SDS loading buffer and incubated for 10min at 70° C; 10μ I of each sample was separated on a SDS PAGE gel, transferred to a nitrocellulose membrane, blocked with 5% BSA and probed with ab24560.

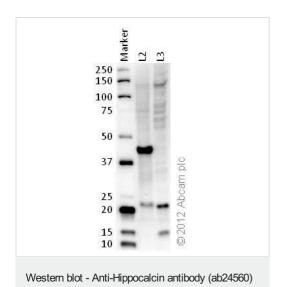
Secondary: Clean blot (HRP conjugate) at 1/1000 dilution.

Band: 23kDa: Hippocalcin; non specific - 60kDa: We are unsure as to the identity of this extra band.



Immunocytochemistry/ Immunofluorescence - Anti-Hippocalcin antibody (ab24560)

ICC/IF image of ab24560 stained SKNSH cells. The cells were 4% formaldehyde 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 ab24560 at 5 μ g/ml overnight at +4°C. The secondary antibody (green) was DyLight® 488 goat anti- rabbit (ab96899) 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) at a concentration of 1.43 μ M.



All lanes: Anti-Hippocalcin antibody (ab24560) at 1 µg/ml

Lane 1: Marker

Lane 2 : Zebrafish brain homogenate (20ug)
Lane 3 : Mouse brain homogenate (20ug)

Secondary

 $\textbf{All lanes:} \ \, \textbf{Goat polyclonal to Rabbit lgG-H\&L-Pre-Adsorbed}$

(HRP) at 1/6000 dilution

Developed using the ECL technique.

Performed under reducing conditions.

Predicted band size: 23 kDa Observed band size: 23 kDa

Exposure time: 1 minute

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

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