abcam

Product datasheet

Anti-Calcium channel L type DHPR alpha 2 subunit/CACNA2D1 antibody [20A] ab2864

★★★★★ 3 Abreviews 35 References 14 Images

Overview

Product name	Anti-Calcium channel L type DHPR alpha 2 subunit/CACNA2D1 antibody [20A]
Description	Mouse monoclonal [20A] to Calcium channel L type DHPR alpha 2 subunit/CACNA2D1
Host species	Mouse
Tested applications	**Suitable for:** Flow Cyt, ICC/IF, ICC, IP, IHC-P, IHC-Fr, WB
Species reactivity	**Reacts with:** Mouse, Rat, Rabbit, Chicken, Guinea pig, Human, Pig
Immunogen	Full length native protein (purified) corresponding to Rabbit Calcium channel L type DHPR alpha 2 subunit/CACNA2D1.
Positive control	WB: rabbit skeletal muscle membrane preparations IHC: rabbit skeletal muscle, mouse brain, rat spinal cord
General notes	This product was previously labelled as Calcium channel L type DHPR alpha 2 subunit

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	Preservative: 0.05% Sodium azide
Purity	Ascites
Primary antibody notes	Voltage-sensitive calcium channels mediate the entry of calcium into many types of excitable cells and thus play a key role in neurotransmitter release and excitation-contraction (E-C) coupling. The 1,4-dihydropyridines (DHPs) are synthetic organic compounds which can be used to identify the L-type calcium channels that are found in all types of vertebrate muscle, neuronal and neuroendocrine cells. The DHP receptor is part of the L-type calcium channel complex and is thought to be the voltage sensor in E-C coupling. The purified DHP receptor isolated from triads is composed of at least four subunits. The alpha-1 subunit contains the binding site for the DHPs and shows high sequence homology to the voltage gated sodium channel. The alpha-2 subunit is

	a large glycoprotein associated with the DHP receptor which was first described in skeletal muscle and is also found in high concentrations in other excitable tissues such as cardiac muscle and brain and in low concentrations in most other tissues studied. The other two subunits that co-purify with the DHP receptor are termed beta and gamma.
Clonality	Monoclonal
Clone number	20A
Isotype	IgG2a

Applications

Our Abpromise guarantee covers the use of **ab2864** in the following tested applications.

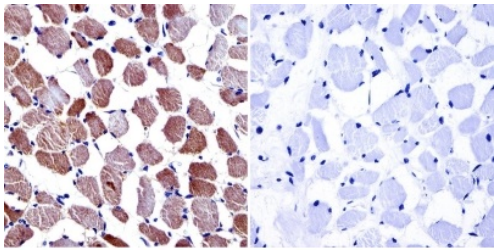
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
Flow Cyt		1/200. ab170191 - Mouse monoclonal IgG2a, is suitable for use as an isotype control with this antibody.
ICC/IF		Use at an assay dependent concentration.
ICC		1/250.
IP		Use at an assay dependent concentration.
IHC-P	★★★★★	1/500. Perform heat mediated antigen retrieval before commencing with IHC staining protocol.
IHC-Fr	★★★★☆	1/500.
WB		1/500. Detects a band of approximately 150 kDa (predicted molecular weight: 123 kDa).

Target

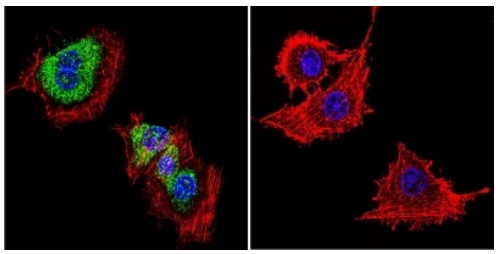
Function	The alpha-2/delta subunit of voltage-dependent calcium channels regulates calcium current density and activation/inactivation kinetics of the calcium channel. Plays an important role in excitation-contraction coupling.
Tissue specificity	Isoform 1 is expressed in skeletal muscle. Isoform 2 is expressed in the central nervous system. Isoform 2, isoform 4 and isoform 5 are expressed in neuroblastoma cells. Isoform 3, isoform 4 and isoform 5 are expressed in the aorta.
Sequence similarities	Belongs to the calcium channel subunit alpha-2/delta family. Contains 1 cache domain. Contains 1 VWFA domain.
Domain	The MIDAS-like motif in the VWFA domain binds divalent metal cations and is required to promote trafficking of the alpha-1 (CACNA1) subunit to the plasma membrane by an integrin-like switch.
Post-translational modifications	Proteolytically processed into subunits alpha-2-1 and delta-1 that are disulfide-linked.
Cellular localization	Membrane.

Images



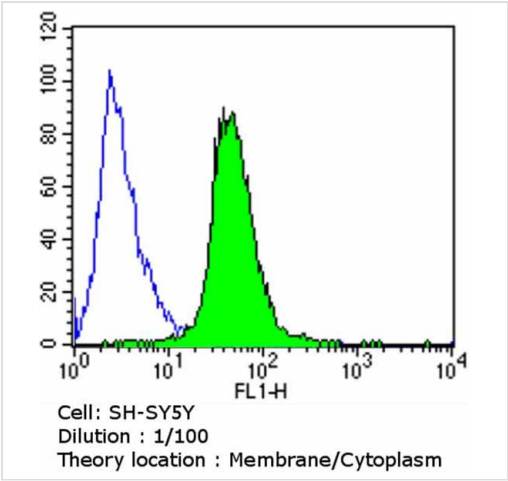
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Calcium channel L type DHPR alpha 2 subunit/CACNA2D1 antibody [20A] (ab2864)

Immunohistochemistry was performed on normal biopsies of deparaffinized human skeletal muscle tissue. To expose target proteins heat induced antigen retrieval was performed using 10mM sodium citrate (pH6.0) buffer and microwaved for 8-15 minutes. Following antigen retrieval tissues were blocked in 3% BSA-PBS for 30 minutes at room temperature. Tissues were then probed at a dilution of 1/100 with a Mouse Monoclonal Antibody recognizing Calcium channel L type DHPR alpha 2 subunit/CACNA2D1 (ab2864) or without primary antibody (negative control) overnight at 4°C in a humidified chamber. Tissues were washed extensively with PBST and endogenous peroxidase activity was quenched with a peroxidase suppressor. Detection was performed using a biotin-conjugated secondary antibody and SA-HRP followed by colorimetric detection using DAB. Tissues were counterstained with hematoxylin and prepped for mounting.



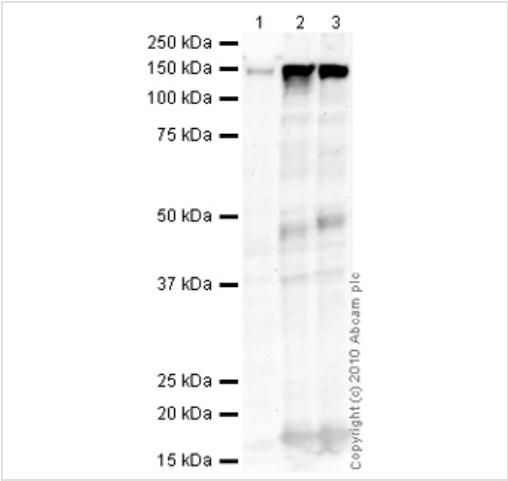
Immunocytochemistry/ Immunofluorescence - Anti-Calcium channel L type DHPR alpha 2 subunit/CACNA2D1 antibody [20A] (ab2864)

Immunofluorescent analysis of Calcium channel L type DHPR alpha 2 subunit/CACNA2D1 in PC12 Cells. Cells were grown on chamber slides and fixed with formaldehyde prior to staining. Cells were probed without (control) or with a Calcium channel L type DHPR alpha 2 subunit/CACNA2D1 monoclonal antibody (ab2864) at a dilution of 1:20 overnight at 4 Cand incubated with a DyLight-488 conjugated secondary antibody. Calcium channel L type DHPR alpha 2 subunit/CACNA2D1 staining (green) F-Actin staining with Phalloidin (red) and nuclei with DAPI (blue) is shown. Images were taken at 60X magnification.



Flow Cytometry - Anti-Calcium channel L type DHPR alpha 2 subunit/CACNA2D1 antibody [20A] (ab2864)

Flow cytometry analysis of Dihydropyridine Receptor alpha 2 showing positive staining in the membrane and cytoplasm of SH-SY5Y cells compared to an isotype control (blue). Cells were harvested and adjusted to a concentration of 1-5x10^6 cells/ml. Cells were then fixed with 2% paraformaldehyde and washed with PBS. Cells were blocked with a 2% solution of BSA-PBS for 30 min at room temperature and incubated with ab2864 at 1:100 for 60 min at room temperature. Cells were then incubated for 40 min at room temperature in the dark using a Dylight 488-conjugated goat anti-mouse IgG (H+L) secondary antibody and re-suspended in PBS for FACS analysis.



Western blot - Anti-Calcium channel L type DHPR alpha 2 subunit/CACNA2D1 antibody [20A] (ab2864)

All lanes : Anti-Calcium channel L type DHPR alpha 2 subunit/CACNA2D1 antibody [20A] (ab2864) at 1/500 dilution

Lane 1 : Spinal Cord (Rat) Tissue Lysate
Lane 2 : SHSY-5Y (Human neuroblastoma cell line) Whole Cell Lysate
Lane 3 : SK N BE (Human neuroblastoma) Whole Cell Lysate

Lysates/proteins at 10 µg per lane.

Secondary
All lanes : Goat Anti-Mouse IgG H&L (HRP) preadsorbed (ab97040) at 1/5000 dilution

Developed using the ECL technique.

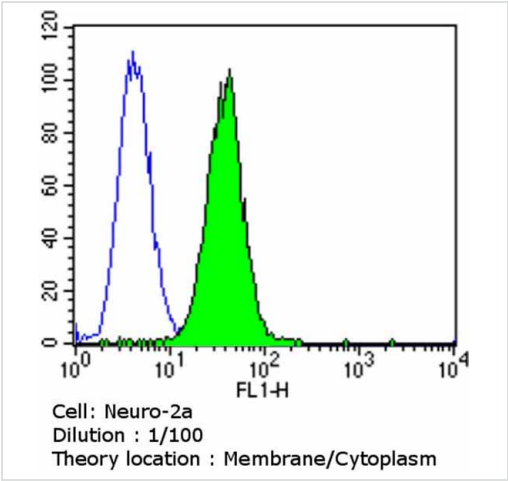
Performed under reducing conditions.

Predicted band size: 123 kDa
Observed band size: 150 kDa
why is the actual band size different from the predicted?
Additional bands at: 18 kDa, 50 kDa. We are unsure as to the identity of these extra bands.

Exposure time: 8 minutes

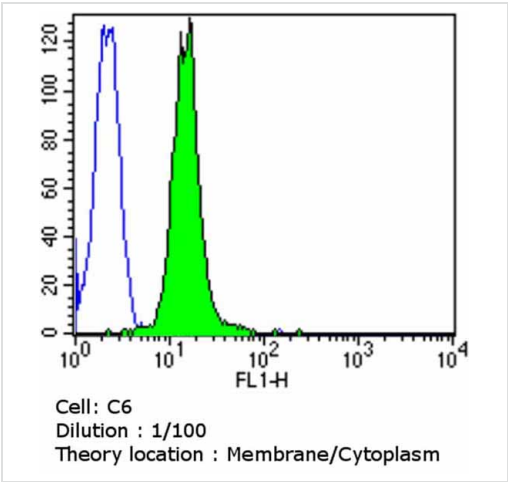
Calcium channel L type DHPR alpha 2 subunit/CACNA2D1

contains a number of potential glycosylation sites (SwissProt) which may explain its migration at a higher molecular weight than predicted.



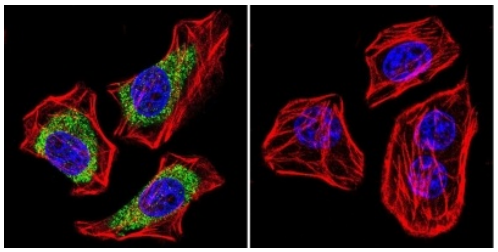
Flow Cytometry - Anti-Calcium channel L type DHPR alpha 2 subunit/CACNA2D1 antibody [20A] (ab2864)

Flow cytometry analysis of Dihydropyridine Receptor alpha 2 showing positive staining in the membrane and cytoplasm of Neuro-2a cells compared to an isotype control (blue). Cells were harvested and adjusted to a concentration of 1-5x10^6 cells/ml. Cells were then fixed with 2% paraformaldehyde and washed with PBS. Cells were blocked with a 2% solution of BSA-PBS for 30 min at room temperature and incubated with a2864 at 1:100 for 60 min at room temperature. Cells were then incubated for 40 min at room temperature in the dark using a Dylight 488-conjugated goat anti-mouse IgG (H+L) secondary antibody and re-suspended in PBS for FACS analysis.



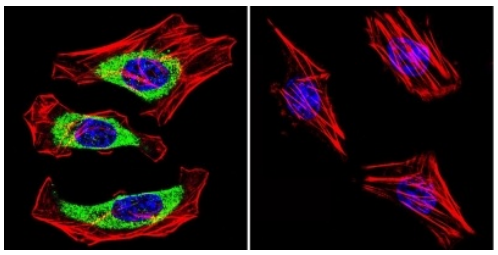
Flow Cytometry - Anti-Calcium channel L type DHPR alpha 2 subunit/CACNA2D1 antibody [20A] (ab2864)

Flow cytometry analysis of Dihydropyridine Receptor alpha 2 showing positive staining in the membrane and cytoplasm of C6 cells compared to an isotype control (blue). Cells were harvested and adjusted to a concentration of 1-5x10^6 cells/ml. Cells were then fixed with 2% paraformaldehyde and washed with PBS. Cells were blocked with a 2% solution of BSA-PBS for 30 min at room temperature and incubated with ab2864 at 1:100 for 60 min at room temperature. Cells were then incubated for 40 min at room temperature in the dark using a Dylight 488-conjugated goat anti-mouse IgG (H+L) secondary antibody and re-suspended in PBS for FACS analysis.



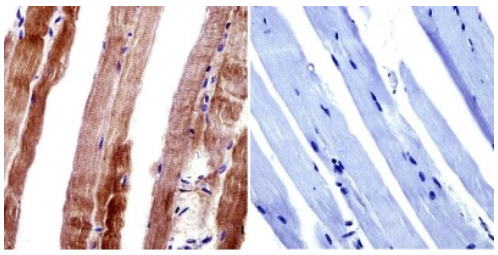
Immunocytochemistry/ Immunofluorescence - Anti-Calcium channel L type DHPR alpha 2 subunit/CACNA2D1 antibody [20A] (ab2864)

Immunofluorescent analysis of Calcium channel L type DHPR alpha 2 subunit/CACNA2D1 in U251 Cells. Cells were grown on chamber slides and fixed with formaldehyde prior to staining. Cells were probed without (control) or with a Calcium channel L type DHPR alpha 2 subunit/CACNA2D1 monoclonal antibody (ab2864) at a dilution of 1:100 overnight at 4 C and incubated with a DyLight-488 conjugated secondary antibody. Calcium channel L type DHPR alpha 2 subunit/CACNA2D1 staining (green) F-Actin staining with Phalloidin (red) and nuclei with DAPI (blue) is shown. Images were taken at 60X magnification.



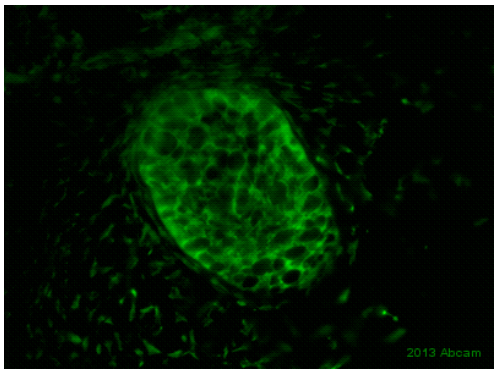
Immunocytochemistry/ Immunofluorescence - Anti-Calcium channel L type DHPR alpha 2 subunit/CACNA2D1 antibody [20A] (ab2864)

Immunofluorescent analysis of Calcium channel L type DHPR alpha 2 subunit/CACNA2D1 in HeLa Cells. Cells were grown on chamber slides and fixed with formaldehyde prior to staining. Cells were probed without (control) or with a Calcium channel L type DHPR alpha 2 subunit/CACNA2D1 monoclonal antibody (ab2864) at a dilution of 1:100 overnight at 4 C and incubated with a DyLight-488 conjugated secondary antibody. Calcium channel L type DHPR alpha 2 subunit/CACNA2D1 staining (green) F-Actin staining with Phalloidin (red) and nuclei with DAPI (blue) is shown. Images were taken at 60X magnification.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Calcium channel L type DHPR alpha 2 subunit/CACNA2D1 antibody [20A] (ab2864)

Immunohistochemistry was performed on normal biopsies of deparaffinized mouse skeletal muscle tissue. To expose target proteins, heat induced antigen retrieval was performed using 10mM sodium citrate (pH6.0) buffer, microwaved for 8-15 minutes. Following antigen retrieval tissues were blocked in 3% BSA-PBS for 30 minutes at room temperature. Tissues were then probed at a dilution of 1:200 with a Mouse Monoclonal Antibody recognizing Calcium channel L type DHPR alpha 2 subunit/CACNA2D1 (ab2864) or without primary antibody (negative control) overnight at 4ºC in a humidified chamber. Tissues were washed extensively with PBST and endogenous peroxidase activity was quenched with a peroxidase suppressor. Detection was performed using a biotin-conjugated secondary antibody and SA-HRP followed by colorimetric detection using DAB. Tissues were counterstained with hematoxylin and prepped for mounting.



Immunohistochemistry (Frozen sections) - Anti-Calcium channel L type DHPR alpha 2 subunit/CACNA2D1 antibody [20A] (ab2864)

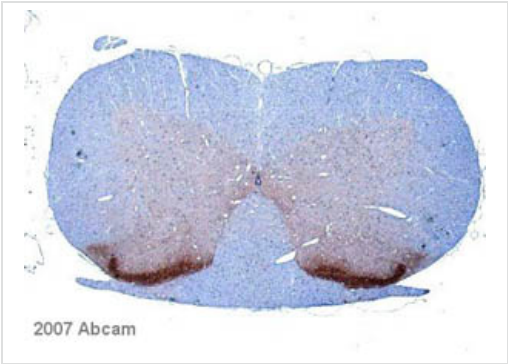
This image is courtesy of an anonymous abreview.

IHC-Fr image of anti-Calcium channel L type DHPR alpha 2 subunit/CACNA2D1 staining with ab2864 on tissue sections from chicken hindbrain. The sections were blocked with 3% BSA for 1 hour at 4°C, before incubation with ab2864 (1/1000 dilution) for 16 hours at 4°C. The secondary was an Alexa-Fluor 488 conjugated goat anti-rabbit polyclonal, used at a 1/1000 dilution.

Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Calcium channel L type DHPR alpha 2 subunit/CACNA2D1 antibody [20A] (ab2864)

This image is courtesy of an Abreview submitted by Mr Carl hobbs

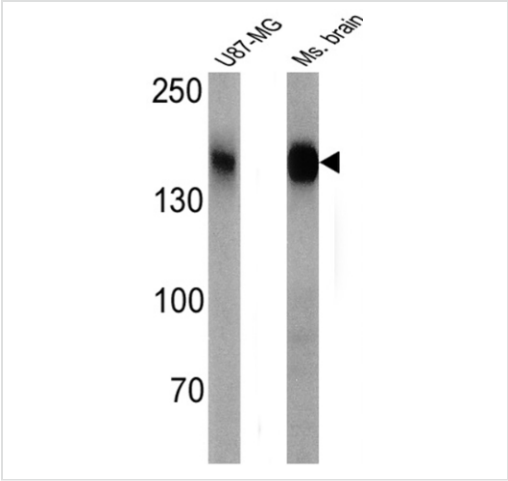
ab2864 at 1/250 staining mouse brain tissue sections by IHC (Formalin/PFA-fixed paraffin-embedded sections). The tissue was formaldehyde fixed and a heat mediated antigen retrieval step was performed. The tissue was incubated with the antibody for 16 hours. A biotinylated goat polyclonal antibody was used as the secondary.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-Calcium channel L type DHPR alpha 2 subunit/CACNA2D1 antibody [20A] (ab2864)

This image is courtesy of an Abreview submitted by Mr Carl hobbs

ab2864 at 1/500 staining rat spinal cord tissue sections by Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections). The tissue was formaldehyde fixed and a heat mediated antigen retrieval step was performed, prior to incubation with the antibody for 16 hours. A biotinylated goat polyclonal antibody was used as the secondary.



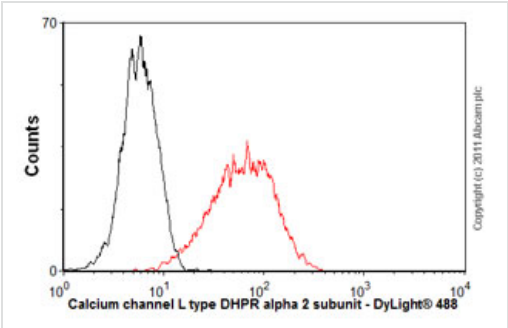
Western blot - Anti-Calcium channel L type DHPR alpha 2 subunit/CACNA2D1 antibody [20A] (ab2864)

All lanes : Anti-Calcium channel L type DHPR alpha 2 subunit/CACNA2D1 antibody [20A] (ab2864) at 1/1000 dilution

Lane 1 : U87-MG cell lysate
Lane 2 : Mouse brain cell lysate

Lysates/proteins at 25 µg per lane.

Predicted band size: 123 kDa
Observed band size: 143 kDa why is the actual band size different from the predicted?



Flow Cytometry - Anti-Calcium channel L type DHPR alpha 2 subunit/CACNA2D1 antibody [20A] (ab2864)

Overlay histogram showing SH-SY5Y cells stained with ab2864 (red line). The cells were fixed with 80% methanol (5 min) and then permeabilized with 0.1% PBS-Tween for 20 min. The cells were then incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions followed by the antibody (ab2864, 1/200 dilution) for 30 min at 22°C. The secondary antibody used was DyLight® 488 goat anti-mouse IgG (H+L) (ab96879) at 1/500 dilution for 30 min at 22°C. Isotype control antibody (black line) was mouse IgG2a [ICIGG2A] (ab91361, 1µg/1x10^6 cells) used under the same conditions. Acquisition of >5,000 events was performed.

Our Abpromise to you: Quality guaranteed and expert technical support

- Replacement or refund for products not performing as stated on the datasheet
- Valid for 12 months from date of delivery
- Response to your inquiry within 24 hours
- We provide support in Chinese, English, French, German, Japanese and Spanish
- Extensive multi-media technical resources to help you
- We investigate all quality concerns to ensure our products perform to the highest standards

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For full details of the Abpromise, please visit https://www.abcam.com/abpromise or contact our technical team.

Terms and conditions

- Guarantee only valid for products bought direct from Abcam or one of our authorized distributors