abcam

Product datasheet

Anti-PSD95 antibody [EPR23124-118] - Synaptic Marker ab238135



Recombinant

RabMAb

*** * * 4 Abreviews 15 References 9 Images

Overview

Product name Anti-PSD95 antibody [EPR23124-118] - Synaptic Marker

Description Rabbit monoclonal [EPR23124-118] to PSD95 - Synaptic Marker

Host species Rabbit

Tested applications Suitable for: WB, IHC-P, IHC-Fr, IP

Unsuitable for: ICC/IF

Species reactivity Reacts with: Mouse, Rat, Human

Immunogen Recombinant fragment. This information is proprietary to Abcam and/or its suppliers.

Positive control WB: Mouse brain, Mouse cerebellum, Rat brain, Human cerebellum and Human brain lysates.

IHC-P: Mouse cerebellum and Rat retina tissues. IP: Mouse brain lysate. IHC-Fr: Mouse and rat

retina tissue.

General notesOur RabMAb[®] technology is a patented hybridoma-based technology for making rabbit

monoclonal antibodies. For details on our patents, please refer to **RabMAb**® **patents**.

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 long

term. Avoid freeze / thaw cycle.

Storage buffer pH: 7.2

Preservative: 0.01% Sodium azide

Constituents: PBS, 40% Glycerol (glycerin, glycerine), 0.05% BSA

Purity Protein A purified

Clonality Monoclonal

Clone number EPR23124-118

Isotype IgG

Applications

1

The Abpromise guarantee

Our **Abpromise guarantee** covers the use of ab238135 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	*** <u>*</u> (2)	1/2000. Predicted molecular weight: 80 kDa.
IHC-P	*** <u>*</u>	1/100.
IHC-Fr		1/100.
IP		1/30.

Application notes

Is unsuitable for ICC/IF.

α	u	L

Function Interacts with the cytoplasmic tail of NMDA receptor subunits and shaker-type potassium

channels. Required for synaptic plasticity associated with NMDA receptor signaling.

Overexpression or depletion of DLG4 changes the ratio of excitatory to inhibitory synapses in hippocampal neurons. May reduce the amplitude of ASIC3 acid-evoked currents by retaining the

channel intracellularly. May regulate the intracellular trafficking of ADR1B.

Tissue specificity Brain.

Sequence similarities Belongs to the MAGUK family.

Contains 1 guanylate kinase-like domain.

Contains 3 PDZ (DHR) domains.

Contains 1 SH3 domain.

Domain The PDZ domain 3 mediates interaction with ADR1B.

The L27 domain near the N-terminus of isoform 2 is required for HGS/HRS-dependent targeting

to postsynaptic density.

Post-translational

modifications

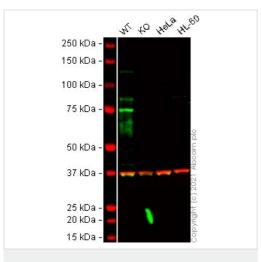
Palmitoylation of isoform 1 is required for targeting to postsynaptic density.

Cellular localization Cell membrane. Cell junction, synapse, postsynaptic cell membrane, postsynaptic density. Cell

projection, axon. Cell junction, synapse. High levels in postsynaptic density of neurons in the forebrain. Also in presynaptic region of inhibitory synapses formed by cerebellar basket cells on

axon hillocks of Purkinje cells.

Images



Western blot - Anti-PSD95 antibody [EPR23124-118] - Synaptic Marker (ab238135)

All lanes : Anti-PSD95 antibody [EPR23124-118] - Synaptic Marker (ab238135) at 1/2000 dilution

Lane 1: Wild-type SH-SY5Y cell lysate

Lane 2: DLG4 knockout SH-SY5Y cell lysate

Lane 3 : HeLa cell lysate

Lane 4 : HL-60 cell lysate

Lysates/proteins at 20 µg per lane.

Secondary

All lanes : Goat anti-Rabbit lgG H&L (IRDye® 800CW) preabsorbed (<u>ab216773</u>) and Goat anti-Mouse lgG H&L (IRDye® 680RD) preabsorbed (<u>ab216776</u>) at 1/20000 dilution

Performed under reducing conditions.

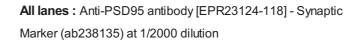
Predicted band size: 80 kDa Observed band size: 75 kDa

Lanes 1 - 4: Merged signal (red and green). Green - ab238135 observed at 75 kDa. Red - loading control <u>ab8245</u> (Mouse anti-GAPDH antibody [6C5]) observed at 37 kDa.

ab238135 was shown to react with PSD95 in wild-type SH-SY5Y cells in Western blot with loss of signal observed in DLG4 knockout cell line ab280043 (DLG4 knockout cell lysate ab280102). Wild-type SH-SY5Y and DLG4 knockout cell lysates were subjected to SDS-PAGE. Membranes were blocked in 3 % milk in TBS-T (0.1 % Tween[®]) before incubation with ab238135 and ab8245 (Mouse anti-GAPDH antibody [6C5]) overnight at 4 °C at a 1 in 2000 dilution 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.



Western blot - Anti-PSD95 antibody [EPR23124-118] - Synaptic Marker (ab238135)



Lane 1: Human cerebellum lysate

Lane 2: Human brain lysate

Lysates/proteins at 20 µg per lane.

Secondary

All lanes : VeriBlot for IP Detection Reagent (HRP) (<u>ab131366</u>) at 1/1000 dilution

Predicted band size: 80 kDa **Observed band size:** 100,75 kDa

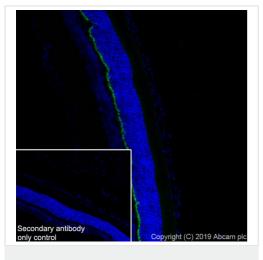
Blocking and diluting buffer and concentration: 5% NFDM/TBST

The molecular weight observed is consistent with what has been described in the literature (PMID: 20118925)

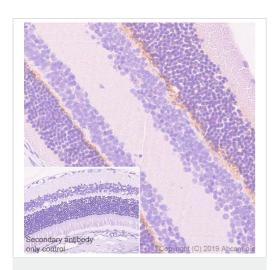
Exposure time: 48 seconds

Immunohistochemical analysis of 4% PFA fixed 0.2% Triton X-100 permeabilized frozen Rat retina tissue labeling VSIG4 with ab238135 at 1/100 dilution followed by **ab150077** AlexaFluor®488 Goat anti-Rabbit secondary at 1/1000 dilution. The nuclear counterstain was DAPI (Blue). Heat mediated antigen retrieval using sodium citrate buffer (10mM citrate pH 6.0 + 0.05% Tween-20).

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody was <u>ab150077</u> AlexaFluor®488 Goat anti-Rabbit secondary at 1/1000 dilution.



Immunohistochemistry (Frozen sections) - Anti-PSD95 antibody [EPR23124-118] - Synaptic Marker (ab238135)



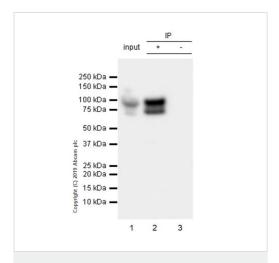
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-PSD95 antibody

[EPR23124-118] - Synaptic Marker (ab238135)

Immunohistochemical analysis of paraffin-embedded Rat retina tissue labeling PSD95 with ab238135 at 1/500 dilution followed by a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101). Positive staining on outer plexiform layer of rat retina is observed. The section was incubated with ab238135 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101).

Heat mediated antigen retrieval with citrate buffer (pH 6.0, epitope retrieval solution 1) for 20mins.



Immunoprecipitation - Anti-PSD95 antibody
[EPR23124-118] - Synaptic Marker (ab238135)

PSD95 was immunoprecipitated from 0.35 mg Mouse brain lysate with ab238135 at 1/30 dilution (2ug in 0.35mg lysates). Western blot was performed on the immunoprecipitate using ab238135 at 1/1000 dilution. VeriBlot for IP Detection Reagent (HRP) (ab131366) was used at 1/5000 dilution.

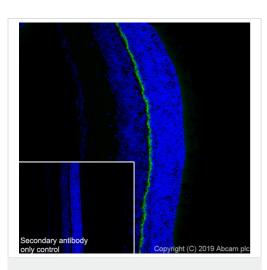
Lane 1: Mouse brain lysate 10µg

Lane 2: ab238135 IP in Mouse brain lysate

Lane 3: Rabbit monoclonal IgG (<u>ab172730</u>) instead of ab238135 in Mouse brain lysate

Blocking and dilution buffer and concentration: 5% NFDM/TBST.

Exposure time: 3 seconds.



Immunohistochemistry (Frozen sections) - Anti-PSD95 antibody [EPR23124-118] - Synaptic Marker (ab238135)

Immunohistochemical analysis of 4% PFA fixed 0.2% Triton X-100 permeabilized frozen Mouse retina tissue labeling VSIG4 with ab238135 at 1/100 (5.76 μ g/ml) dilution followed by **ab150077** AlexaFluor®488 Goat anti-Rabbit secondary at 1/1000 (2 μ g/ml) dilution. The nuclear counterstain was DAPI (Blue). Heat mediated antigen retrieval using sodium citrate buffer (10mM citrate pH 6.0 + 0.05% Tween-20).

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody was <u>ab150077</u> AlexaFluor®488 Goat anti-Rabbit secondary at 1/1000 (2 µg/ml) dilution.



Western blot - Anti-PSD95 antibody [EPR23124-118] - Synaptic Marker (ab238135)

All lanes : Anti-PSD95 antibody [EPR23124-118] - Synaptic Marker (ab238135) at 1/2000 dilution

Lane 1: Mouse brain lysate

Lane 2: Mouse cerebellum lysate

Lane 3: Rat brain lysate

Lysates/proteins at 20 µg per lane.

Secondary

All lanes : Goat Anti-Rabbit lgG H&L (HRP) (<u>ab97051</u>) at 1/100000 dilution

Predicted band size: 80 kDa **Observed band size:** 100,75 kDa

Blocking and diluting buffer and concentration: 5% NFDM/TBST

The molecular weight observed is consistent with what has been described in the literature (PMID: 20118925).

Exposure time: 15 seconds

Secondary antibody only control Copyright (C) 2019 Abcam pic

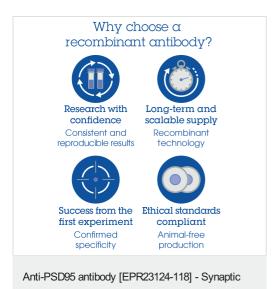
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-PSD95 antibody

[EPR23124-118] - Synaptic Marker (ab238135)

Immunohistochemical analysis of paraffin-embedded Mouse cerebellum tissue labeling PSD95 with ab238135 at 1/100 dilution followed by a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101). Positive staining on basket cells of mouse cerebellum is observed. The section was incubated with ab238135 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND® RX instrument. Counterstained with Hematoxylin.

Secondary antibody only control: Secondary antibody is a ready to use Rabbit specific IHC polymer detection kit HRP/DAB (ab209101).

Heat mediated antigen retrieval with citrate buffer (pH 6.0, epitope retrieval solution 1) for 20mins.



Marker (ab238135)

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