abcam

Product datasheet

Anti-PSD95 antibody - Synaptic Marker ab18258

*** * * * 39 Abreviews 315 References 9 Images

Overview

Product name Anti-PSD95 antibody - Synaptic Marker

Description Rabbit polyclonal to PSD95 - Synaptic Marker

Host species Rabbit

Tested applications Suitable for: ICC/IF, WB, IHC-P

Species reactivity Reacts with: Mouse, Rat, Human

Predicted to work with: Zebrafish, Cynomolgus monkey, Common marmoset

Immunogen Synthetic peptide corresponding to Mouse PSD95 aa 50-150 (internal sequence) conjugated to

keyhole limpet haemocyanin (Cysteine residue).

(Peptide available as ab18661)

Positive control WB: Mouse & rat brain tissue lysate, SHSY5Y whole cell lysate . ICC/IF: SH-SY5Y cells , Primary

hippocampal rat neurons/glia, (obtained from Neuromics, cat. no. PC35101), DIV14. IHC-P:

Mouse & rat 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.

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Purity Immunogen affinity purified

Clonality Polyclonal

Isotype IgG

Applications

The Abpromise guarantee Our Abpromise guarantee covers the use of ab18258 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/IF	★★★★ ★ <u>(5)</u>	Use a concentration of 1 - 5 μg/ml.
WB	★★★★ ★ (14)	Use a concentration of 1 - 3 µg/ml. Detects a band of approximately 85 kDa (predicted molecular weight: 80 kDa). Abcam recommends using 3% milk as the blocking agent.
IHC-P	**** <u>(7)</u>	Use at an assay dependent concentration.

T	a	rg	et

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

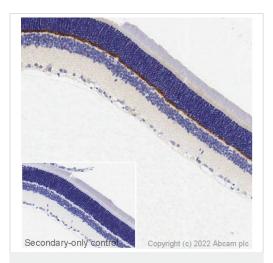
Palmitoylation of isoform 1 is required for targeting to postsynaptic density.

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



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-PSD95 antibody - Synaptic Marker (ab18258)

ab18258 ab7291

Immunocytochemistry/ Immunofluorescence - Anti-PSD95 antibody - Synaptic Marker (ab18258)

IHC image of PSD95 staining in a section of formalin-fixed paraffinembedded normal rat eye performed on a Leica BOND™ system using the standard protocol F. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH6, epitope retrieval solution 1) for 20mins. The section was then incubated with ab18258, 1ug/ml, for 15 mins at room temperature and detected using an HRP conjugated compact polymer system. DAB was used as the chromogen. The section was then counterstained with haematoxylin and mounted with DPX. The inset secondary-only control image is taken from an identical assay without primary antibody.

For other IHC staining systems (automated and non-automated) customers should optimize variable parameters such as antigen retrieval conditions, primary antibody concentration and antibody incubation times.

ab18258 staining PSD95 in primary hippocampal rat neurons/glia, (obtained from Neuromics, cat. no. PC35101), DIV14. cells. The cells were fixed with 100% methanol (5 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 ab18258 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 IgG - H&L (Alexa Fluor® 488), pre-adsorbed at 1/1000 dilution (shown in green) and ab150120, Goat polyclonal Secondary Antibody to Mouse IgG - H&L (Alexa Fluor® 594), 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 4% paraformaldehyde (10 min).

Image was acquired with a confocal microscope (Leica-Microsystems TCS SP8) and a single confocal section is shown.



Western blot - Anti-PSD95 antibody - Synaptic Marker (ab18258) All lanes : Anti-PSD95 antibody - Synaptic Marker (ab18258) at 1 $\mu g/ml$

Lane 1: Mouse brain tissue lysate at 10 μg **Lane 2**: Human brain tissue lysate at 20 μg **Lane 3**: SHSY5Y whole cell lysate at 20 μg

Secondary

All lanes : Goat polyclonal to Rabbit lgG - H&L - Pre-Adsorbed

(HRP) at 1/50000 dilution

Predicted band size: 80 kDa **Observed band size:** 85 kDa

Exposure time: 8 minutes

Gel type: MOPS

Blocking buffer: 1% milk

Disconning Sanon 170 min

All lanes: Anti-PSD95 antibody - Synaptic Marker (ab18258) at 1

µg/ml

Lane 1 : Brain (Mouse) Tissue Lysate (<u>ab27253</u>)

Lane 2: Brain (Rat) Tissue Lysate (ab7942)

Lysates/proteins at 10 µg per lane.

100 kDa — 75 kDa — 50 kDa — 37 kDa — 35 kDa — 25 kDa — 25 kDa — 37 kDa — 37 kDa — 37 kDa — 38 kDa — 38 kDa — 38 kDa

250 kDa =

150 kDa -

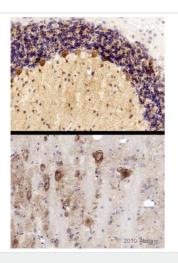
Western blot - Anti-PSD95 antibody - Synaptic Marker (ab18258)

Secondary

All lanes : Rabbit IgG secondary antibody (<u>ab28446</u>) at 1/10000 dilution

Performed under reducing conditions.

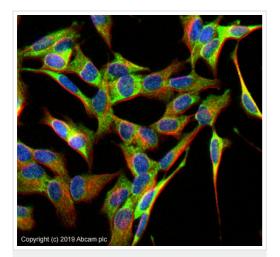
Predicted band size: 80 kDa **Observed band size:** 85 kDa



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-PSD95 antibody - Synaptic Marker (ab18258)

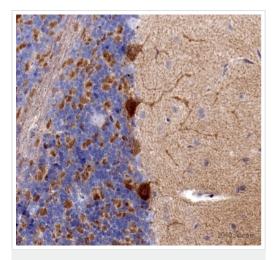
This image is courtesy of an abreview submitted by Carl Hobbs, King's College London, United Kingdom

ab18258 (1/500) staining PSD95 in paraffin-embedded mouse cerebellum (top) and medulla (bottom) tissue, showing positive staining to the synaptic regions of the brain. Tissue was fixed in formaldehyde, blocking performed using 1% BSA (10 minutes/RT) and heat mediated antigen retrieval performed before staining. The secondary antibody (1/200) was goat anti rabbit lgG conjugated to Biotin. For further experimental details, please refer to abreview.



Immunocytochemistry/ Immunofluorescence - Anti-PSD95 antibody - Synaptic Marker (ab18258)

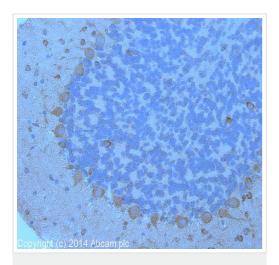
ab18258 staining PSD95 in SH-SY5Y cells. The cells were fixed with 100% methanol (5 min) at room temperature, permeabilized with 0.1% Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated with the antibody ab18258 at 5μg/ml and ab7291 (Mouse monoclonal to alpha Tubulin - Loading Control) used at a 1/1000 dilution overnight at +4°C. The secondary antibodies were ab150081, Goat Anti-Rabbit lgG H&L (Alexa Fluor® 488) preadsorbed, (pseudo-colored green) and ab150120, Goat polyclonal Secondary Antibody to Mouse lgG - H&L (Alexa Fluor® 594) preadsorbed, (colored red), both used at a 1/1000 dilution for 1 hour at room temperature. DAPI was used to stain the cell nuclei (colored blue) at a concentration of 1.43 μM for 1hour at room temperature.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-PSD95 antibody - Synaptic Marker (ab18258)

This image is courtesy of an abreview submitted by Carl Hobbs, King's College London, United Kingdom.

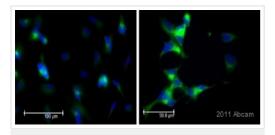
ab18258 (1/1000) staining PSD9 in paraffin-embedded rat cerebellum. Tissue was fixed in formaldehyde, blocking performed using 1% BSA (10 minutes/RT) and heat mediated antigen retrieval performed before staining. The secondary antibody (1/200) was goat anti rabbit lgG conjugated to Biotin. For further experimental details, please refer to abreview.



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-PSD95 antibody - Synaptic Marker (ab18258)

IHC image of PSD95 staining in mouse brain formalin fixed paraffin embedded tissue section, performed on a Leica Bond system using the standard protocol F. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH6, epitope retrieval solution 1) for 20 minutes. The section was then incubated with ab18258, 1 μ g/ml, for 15 minutes at room temperature and detected using an HRP conjugated compact polymer system. DAB was used as the chromogen. The section was then counterstained with haematoxylin and mounted with DPX.

For other IHC staining systems (automated and non-automated) customers should optimize variable parameters such as antigen retrieval conditions, primary antibody concentration and antibody incubation times.



Immunocytochemistry/ Immunofluorescence - Anti-PSD95 antibody - Synaptic Marker (ab18258) Image courtesy of an anonymous Abreview.

ab18258 staining PSD95 in human SH-SY5Y cells by Immunocytochemistry/ Immunofluorescence. Cells were fixed in paraformaldehyde, blocked with 10% serum for 20 minutes at 24°C, then incubated with ab18258 at a 1/1000 dilution for 16 hours at 4°C. The secondary used was an Alexa-Fluor® 488 conjugated donkey anti-rabbit polyclonal used at a 1/1000 dilution. Counterstained with Hoechst 33258 (blue).

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