abcam

Product datasheet

Anti-TMEM119 antibody [28-3] - BSA and Azide free ab234501

KO VALIDATED Recombinant RabMAb

1 Abreviews 13 Images

Overview

Product name	Anti-TMEM119 antibody [28-3] - BSA and Azide free
Description	Rabbit monoclonal [28-3] to TMEM119 - BSA and Azide free
Host species	Rabbit
Tested applications	**Suitable for:** IHC-FoFr, IHC-Fr, IHC-P
Species reactivity	**Reacts with:** Mouse **Does not react with:** Rat, Human
Immunogen	Recombinant fragment (GST-tag) within Mouse TMEM119 aa 100 to the C-terminus (intracellular). The exact sequence is proprietary.
Positive control	IHC-Fr: mouse brain, mouse cerebrum. IHC-P: FFPE mouse brain. Mouse brain cerebral cortex, hippocampus and cerebellum stain positive for Tmem119. Please note that Tmem119 expression is seen after postnatal day (P) 14 in mouse brain. IHC-FoFr: Mouse cerebrum.
General notes	ab234501 is the carrier-free version of **ab209064**. This Tmem119 antibody has been knockout validated in IHC, meaning it demonstrated the expected staining in wild type mouse brain sections and no staining was observed in Tmem119 knockout mouse brain sections. The data are shown on this datasheet. To detect mouse Tmem119 by flow cytometry, we recommend using **ab210405**. To detect human TMEM119 by IHC, we recommend using **ab185333**. **The 28-3 clone to mouse Tmem119 is exclusively manufactured and sold by Abcam.** **IHC-Frozen protocol advice:** For immunohistochemistry on frozen sections, it is recommended that a high concentration of Triton X-100 (0.5%) is used during permeabilization and antibody incubation steps. This may increase the proportion of microglia that stain positive for Tmem119. If you require this antibody in a particular buffer formulation or a particular conjugate for your experiments, please contact **orders@abcam.com** or you can find further information **here**. Our **carrier-free** antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. The carrier-free buffer and high concentration allow for increased conjugation efficiency. This conjugation-ready format is designed for use with fluorochromes, metal isotopes, oligonucleotides, and enzymes, which makes them ideal for antibody labelling, functional and cell-

based assays, flow-based assays (e.g. mass cytometry) and Multiplex Imaging applications.

Use our **conjugation kits** for antibody conjugates that are ready-to-use in as little as 20 minutes with <1 minute hands-on-time and 100% antibody recovery: available for fluorescent dyes, HRP, biotin and gold.

This product is compatible with the Maxpar® Antibody Labeling Kit from Fluidigm, without the need for antibody preparation. Maxpar® is a trademark of Fluidigm Canada Inc.

Properties

Form	Liquid
Storage instructions	Shipped at 4°C. Store at +4°C. Do Not Freeze.
Storage buffer	pH: 7.2 Constituent: PBS
Carrier free	Yes
Purity	Protein A purified
Clonality	Monoclonal
Clone number	28-3
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab234501 in the following tested applications.

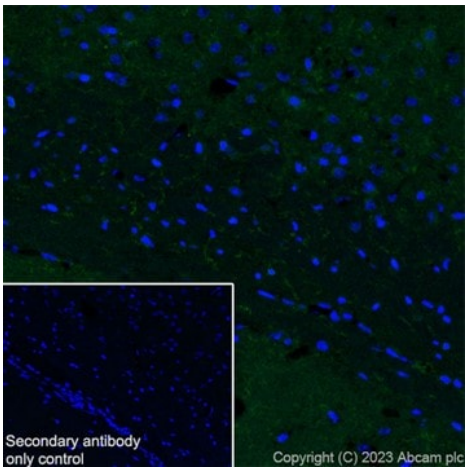
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
IHC-FoFr		Use at an assay dependent concentration. Perform heat mediated antigen retrieval with citrate buffer pH 6. Incubate the section with primary antibody at 4 ? overnight.
IHC-Fr		Use at an assay dependent concentration. We recommend using 0.3-0.5% Triton X-100. Perform heat mediated antigen retrieval before IHC-Fr staining protocol, if the signal is too weak.
IHC-P		Use at an assay dependent concentration. Perform heat mediated antigen retrieval before commencing with IHC staining protocol. Tris-EDTA buffer preferred.

Target

Cellular localization Membrane; Single-pass type I membrane protein

Images

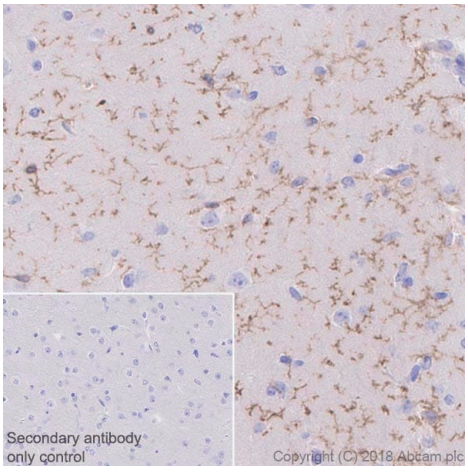


Immunohistochemistry (PFA perfusion fixed frozen sections) - Anti-TMEM119 antibody [28-3] - BSA and Azide free (ab234501)

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab209064**).

IHC image of TMEM119 staining in a section of Mouse cerebrum using **ab209064** at 1:50 dilution.The section was fixed with 4% PFA then permeabilized with 0.2% Triton X-100. The secondary antibody was **ab150081**, Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed used at 1:1000 dilution. Nuclear DNA was labelled with DAPI (shown in blue). Heat mediated antigen retrieval using sodium citrate buffer (10mM citrate pH 6.0 + 0.05% Tween-20). Secondary antibody only control: PBS instead of the primary antibody.

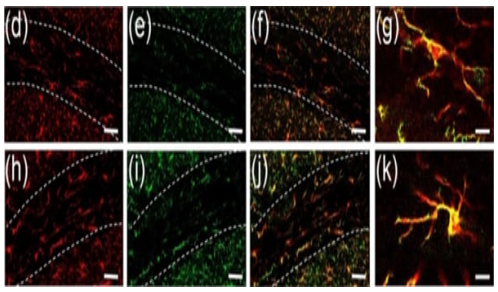
Positive staining on mouse cerebrum.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-TMEM119 antibody [28-3] - BSA and Azide free (ab234501)

ab209064 at 1:2000 staining TMEM119 antibody in mouse cerebrum tissue by immunohistochemistry (FFPE). Immunohistochemical analysis of paraffin-embedded mouse cerebrum tissue labeling TMEM119 with **ab209064** at 1/2000 dilution followed by Goat Anti-Rabbit IgG H&L (HRP). Positive staining on glial cells in mouse cerebrum is observed. Perform heat mediated antigen retrieval using **ab93684** (Tris/EDTA buffer, pH 9.0) before commencing with IHC staining protocol. Counter stained with hematoxylin.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab209064**).



Immunohistochemistry (Frozen sections) - Anti-TMEM119 antibody [28-3] - BSA and Azide free (ab234501)

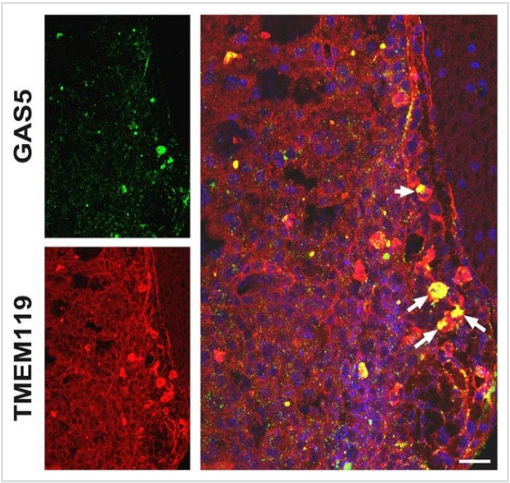
Image from Manso Y et al. Glia. 2018;66(1):34-46. Fig 4.; doi: 10.1002/glia.23190.

Representative images of sham (d–g) and hypoperfusion (h–k) at 12 weeks post-surgery are shown to illustrate Iba-1 immunostaining in sham (d) and hypoperfused (h); TMEM119 immunostaining in sham (e) and hypoperfused (i) and then Iba-1/TMEM119 co-localisation in sham (f,g) and hypoperfused (j,k) white matter. All Iba-1$^+$ cells in both sham and hypoperfused cohorts were also TMEM119$^+$ indicating that the cells in the corpus callosum were resident microglia. Scale bars; d-f and h-j are 50µm, g and k 10 µm. The number of microglial cells significantly correlated with nodal gap length.

Free floating cryo-preserved sections cut at 30 µm thickness. Sections were incubated with the primary antibodies (anti-Iba-1

(1/100) and anti-TMEM119 (1/500, **ab209064**)) overnight at 4°C. Sections were stained at the outset with haematoxylin and eosin to determine the presence and absence of ischemic neuronal perikaryal damage as part of the inclusion/exclusion criteria.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab209064**).



Immunohistochemistry (Frozen sections) - Anti-TMEM119 antibody [28-3] - BSA and Azide free (ab234501)

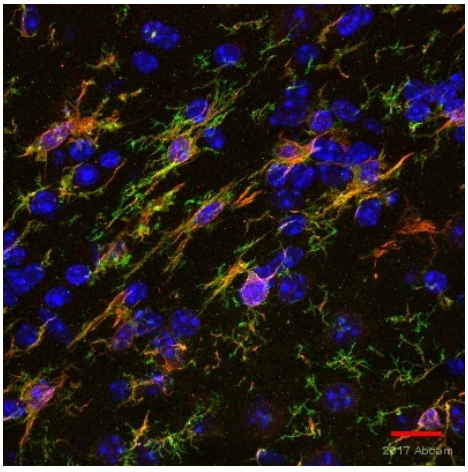
Image from Sun D et al. EMBO Rep. 2017;18(10):1801-1816. Fig EV3.; doi: 10.15252/embr.201643668.

Representative FISH analysis of GAS5 (green) co-stained with **ab209064** (red) in spinal cord sections from EAE mice at 30 dpi. Arrows indicate $GAS5^{+}TMEM119^{+}$ cells. Scale bars = 25 μm.

Female C57BL/6 mice (6–8 weeks) were deeply anesthetized with 3% chloral hydrate and a laminectomy was performed. After fixing the spine, 1 μl of 1% lysolecithin in a 0.9% sodium chloride solution was injected into the dorsal funiculus at the level of the T11–T12 vertebrae. The day of lysolecithin injection was designated day 0 (0 dpi). The spinal cord around the injection point was isolated and cut into serial cryosections.

Tissue sections were fixed, permeabilized, and incubated with the primary antibody overnight at 4°C, followed by 2 h of incubation with TRITC- or FITC-conjugated secondary antibodies. Then, the samples were counterstained with Hoechst 33342.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab209064**).



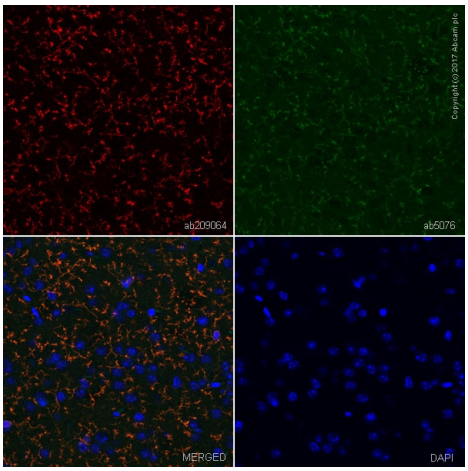
Immunohistochemistry (Frozen sections) - Anti-TMEM119 antibody [28-3] - BSA and Azide free (ab234501)

This image is courtesy of an anonymous Abreview.

ab209064 staining TMEM119 in Mouse corpus callosum sections by Immunohistochemistry (IHC-Fr - frozen sections). Tissue was fixed with paraformaldehyde and blocked with 0.5% BSA for 1 hour at 23°C. Samples were incubated with primary antibody at 1.4µg/ml for 18 hours at 4°C. An Alexa Fluor® 488 -conjugated Donkey anti-rabbit IgG polyclonal was used as the secondary antibody.

TMEM119 (green), Iba1 (red) and DAPI (blue)

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab209064**).

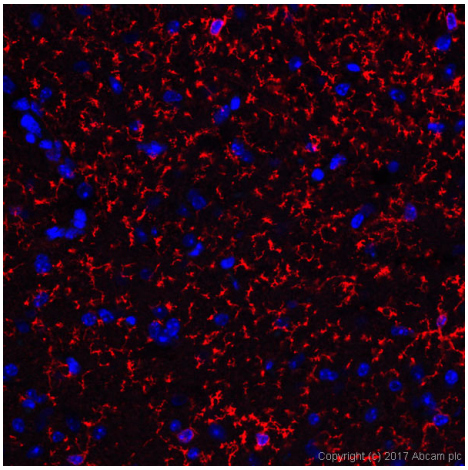


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-TMEM119 antibody [28-3] - BSA and Azide free (ab234501)

IHC image of TMEM119 and Iba1 co-staining in a section of formalin-fixed paraffin-embedded normal mouse brain. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH6) in a Dako Pascal pressure cooker using the standard factory-set regime. Non-specific protein-protein interactions were then blocked in PBS containing 0.5% (v/v) Triton X-100, 0.3 M (w/v) glycine and 1% (w/v) BSA for 1 h at room temperature. The section was then incubated overnight at +4°C in TBS containing 0.5% (v/v) Triton X-100 and 1% (w/v) BSA with **ab209064** at 1 µg/ml and **ab5076** at 5 µg/ml. The secondary antibodies were **ab150087** (shown in red) and **ab150133** (shown in green) used at 2 µg/ml for 1 hour at room temperature. Nuclear DNA was labelled with DAPI (shown in blue). The section was then mounted using Fluoromount®. Images were taken with a confocal microscope (Leica-Microsystems, TCS SP8).

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

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab209064**).



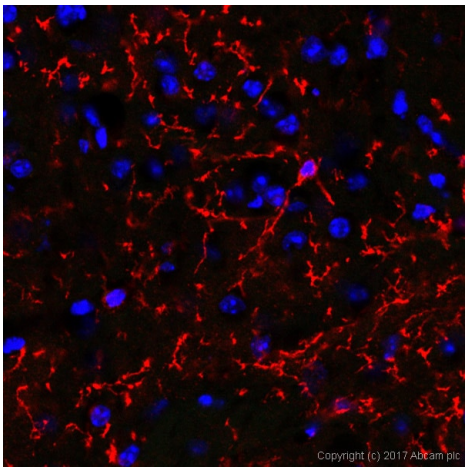
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-TMEM119 antibody [28-3] - BSA and Azide free (ab234501)

IHC image of TMEM119 staining in a section of formalin fixed, paraffin embedded normal mouse brain. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH6) in a Dako Pascal pressure cooker using the standard factory-set regime. Non-specific protein-protein interactions were then blocked in PBS containing 0.5% (v/v) Triton X-100, 0.3 M (w/v) glycine and 1% (w/v) BSA for 1 h at room temperature. The section was then incubated overnight at +4°C in PBS containing 0.5% (v/v) Triton X-100 and 1% (w/v) BSA with **ab209064** at 0.1 µg/ml. The secondary antibody was **ab150087** (shown in red) used at 2 µg/ml for 1 hour at room temperature. Nuclear DNA was labelled with DAPI (shown in blue). The section was then mounted using Fluoromount®. The image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).

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

This data was developed using the same antibody clone in a

different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab209064**).

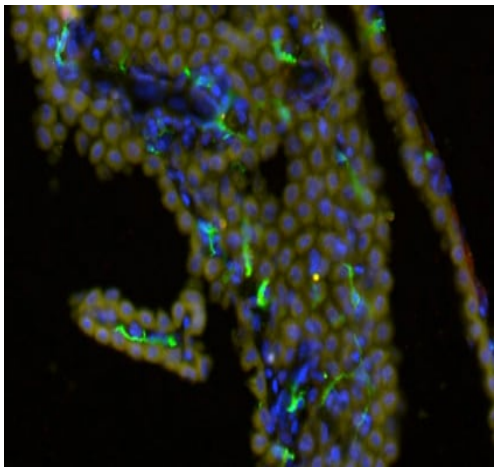


Immunohistochemistry (Frozen sections) - Anti-TMEM119 antibody [28-3] - BSA and Azide free (ab234501)

IHC image of TMEM119 staining in a section of frozen normal mouse brain. No antigen retrieval step was performed prior to staining. Non-specific protein-protein interactions were then blocked in PBS containing 0.5% (v/v) Triton X-100, 0.3 M (w/v) glycine and 1% (w/v) BSA for 1 h at room temperature. The section was then incubated overnight at +4°C in PBS containing 0.5% (v/v) Triton X-100 and 1% (w/v) BSA with **ab209064** at 0.5 µg/ml. The secondary antibody was **ab150087** (shown in red) used at 2 µg/ml for 1 hour at room temperature. Nuclear DNA was labelled with DAPI (shown in blue). The section was then mounted using Fluoromount®. The image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).

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

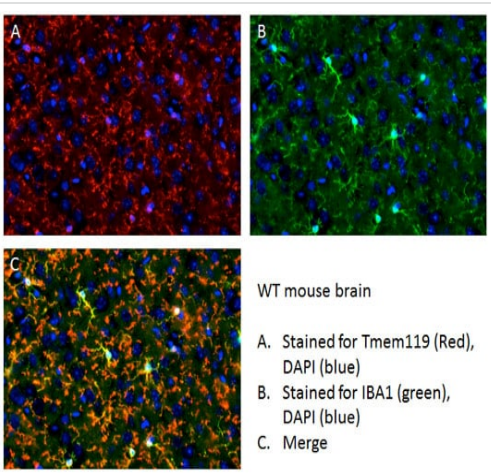
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab209064**).



Immunohistochemistry (Frozen sections) - Anti-TMEM119 antibody [28-3] - BSA and Azide free (ab234501)

Normal mouse choroid plexus, stained for TMEM119 (red), Iba1 (green) and DAPI (blue). Choroid plexus macrophages are positive for Iba1 and negative for TMEM119. Samples were baked onto slides for 10 minutes at 60°C, rehydrated with PBS and blocked with blocking buffer (10% serum in PBST). **ab209064** at a concentration of 1 µg/mL was incubated with the sample overnight at 4°C. Slides were washed with PBS and a goat anti-rabbit Alexa Fluor 488® was used as the secondary antibody at a concentration of 4 µg/mL.

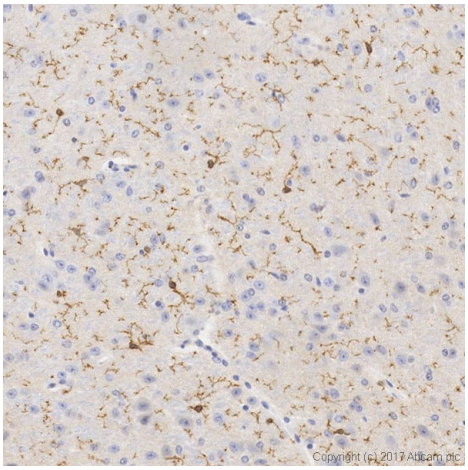
This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab209064**).



Immunohistochemistry (Frozen sections) - Anti-TMEM119 antibody [28-3] - BSA and Azide free (ab234501)

This IHC data was generated using the same anti-TMEM119 antibody clone, 28-3, in a different buffer formulation (cat# **ab209064**).

Normal (WT) mouse brain, stained for TMEM119 (red), Iba1 (green) and DAPI (blue). Samples were baked onto slides for 10 minutes at 60^{O}C, rehydrated with PBS and blocked with blocking buffer (10% serum in PBST). **ab209064** at a concentration of 1 µg/mL was incubated with the sample overnight at 4^{O}C. Slides were washed with PBS and a goat anti-rabbit Alexa Fluor 488® was used as the secondary antibody at a concentration of 4 µg/mL.

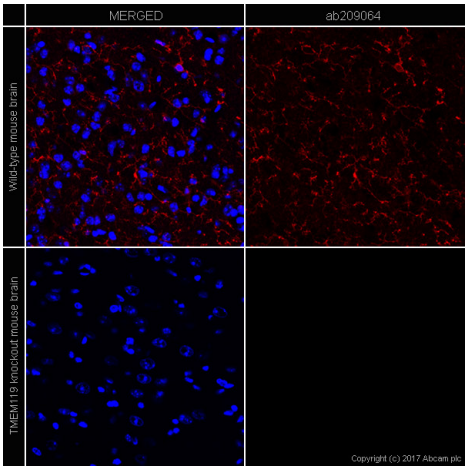


Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-TMEM119 antibody [28-3] - BSA and Azide free (ab234501)

This IHC data was generated using the same anti-TMEM119 antibody clone, 28-3, in a different buffer formulation (cat# **ab209064**).

IHC image of TMEM119 staining in a section of formalin fixed, paraffin embedded normal mouse brain, performed on a Leica Bond™ system using the standard protocol B. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH6, epitope retrieval solution 1) for 20 mins. The section was then incubated with **ab209064**, 0.5 µg/ml, for 15 mins at room temperature. A goat anti-Rabbit biotinylated secondary antibody was used to detect the primary, and visualized using an HRP conjugated ABC 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.

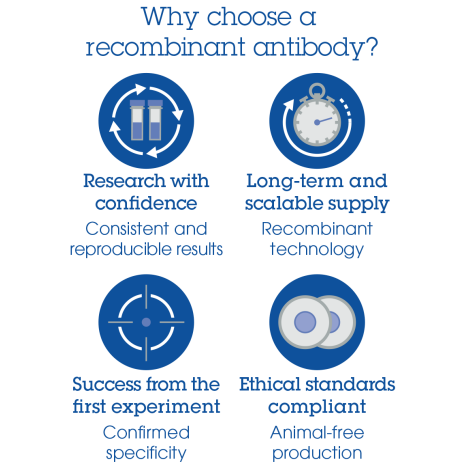


Immunohistochemistry (Frozen sections) - Anti-TMEM119 antibody [28-3] - BSA and Azide free (ab234501)

This IHC data was generated using the same anti-TMEM119 antibody clone, 28-3, in a different buffer formulation (cat# **ab209064**).

IHC image of TMEM119 staining in a section of frozen normal mouse brain wild type (upper panel) and TMEM119 knockout (lower panel). No antigen retrieval step was performed prior to staining. Non-specific protein-protein interactions were then blocked in PBS containing 0.5% (v/v) Triton X-100, 0.3 M (w/v) glycine and 1% (w/v) BSA for 1 h at room temperature. The section was then incubated overnight at +4°C in PBS containing 0.5% (v/v) Triton X-100 and 1% (w/v) BSA with **ab209064** at 0.5 µg/ml. The secondary antibody was **ab150087** (shown in red) used at 2 µg/ml for 1 hour at room temperature. Nuclear DNA was labelled with DAPI (shown in blue). The section was then mounted using Fluoromount®. Images were taken with a confocal microscope (Leica-Microsystems, TCS SP8).

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



Anti-TMEM119 antibody [28-3] - BSA and Azide free (ab234501)

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