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Product datasheet

Anti-TMEM119 antibody [28-3] - Microglial marker ab209064

KO 評価済 リコンビナント RabMAb

23 Abreviews 115 References 画像数 13

製品の概要

製品名	Anti-TMEM119 antibody [28-3] - Microglial marker
製品の詳細	Rabbit monoclonal [28-3] to TMEM119 - Microglial marker
由来種	Rabbit
特異性	This antibody recognizes mouse Tmem119, a transmembrane protein that has been reported to be a highly specific microglia marker that is not expressed by macrophages or other immune or neural cell types (Bennett et al., 2016).
アプリケーション	**適用あり:** IHC-Fr, IHC-P, IHC-FoFr
種交差性	**交差種:** Mouse **非交差種:** Rat, Human
免疫原	Recombinant fragment (GST-tag) within Mouse TMEM119 aa 100 to the C-terminus (intracellular). The exact sequence is proprietary.
ポジティブ・コントロール	IHC-Fr: mouse brain, mouse corpus callosum, mouse choroid plexus, spinal cord sections from EAE mice, mouse cerebrum. IHC-P: FFPE mouse brain, mouse cerebrum. IHC-FrFl: Mouse brain IHC-FoFr: Mouse cerebrum.
特記事項	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. This data is shown on this datasheet in the images section. 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. Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to **RabMAb® patents**.

製品の特性

製品の状態	Liquid

保存方法	Shipped at 4°C. Store at +4°C. Do Not Freeze.
バッファー	pH: 7.2 Preservative: 0.02% Sodium azide Constituents: PBS, 6.97% L-Arginine
精製度	Protein A purified
ポリ/モノ	モノクローナル
クローン名	28-3
アイソタイプ	IgG

アプリケーション

The Abpromise guarantee **Abpromise保証は、** 次のテスト済みアプリケーションにおけるab209064の使用に適用されます

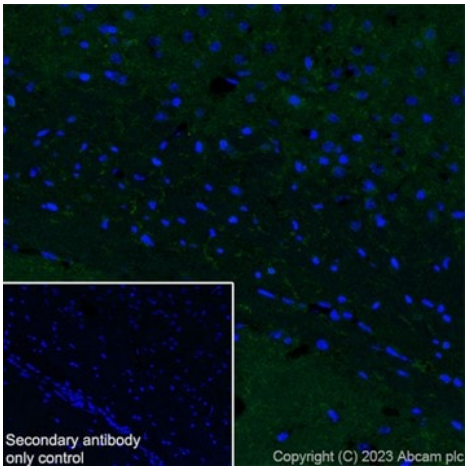
アプリケーションノートには、推奨の開始希釈率がありますが、適切な希釈率につきましてはご検討ください。

アプリケーション	Abreviews	特記事項
IHC-Fr	★★★★☆ (7)	Use a concentration of 0.5 - 1 µg/ml. 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	★★★★☆ (7)	Use a concentration of 0.1 - 0.5 µg/ml. Perform heat mediated antigen retrieval before commencing with IHC staining protocol. Tris-EDTA buffer preferred.
IHC-FoFr	★★★★★ (1)	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.

ターゲット情報

細胞内局在	Membrane; Single-pass type I membrane protein

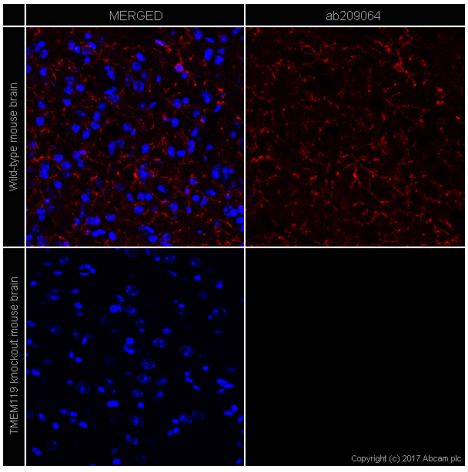
画像



Immunohistochemistry (PFA perfusion fixed frozen sections) - Anti-TMEM119 antibody [28-3] - Microglial marker (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.

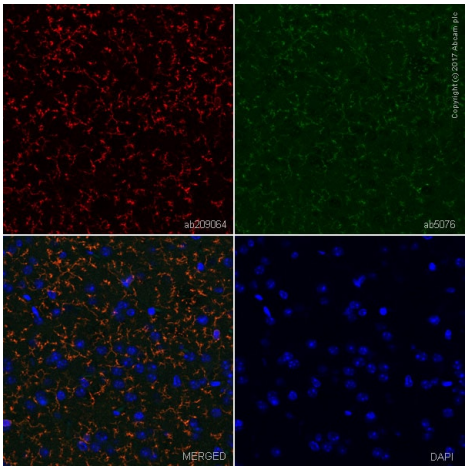
Positive staining on mouse cerebrum.



Immunohistochemistry (Frozen sections) - Anti-TMEM119 antibody [28-3] - Microglial marker (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).

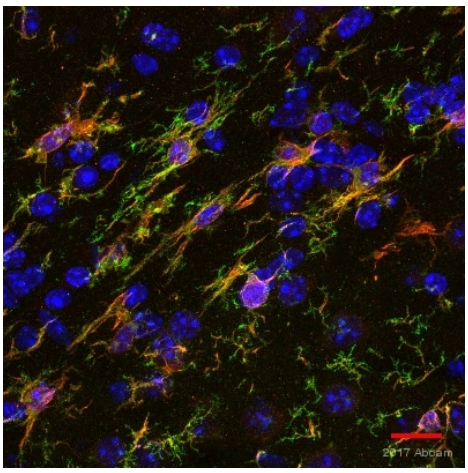
For other IHC staining systems (automated and non-automated), customers should optimize variable parameters such as antigen retrieval conditions, antibody concentrations and incubation times.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-TMEM119 antibody [28-3] - Microglial marker (ab209064)

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.

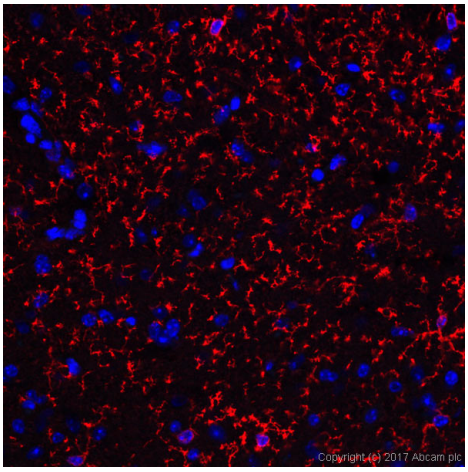


Immunohistochemistry (Frozen sections) - Anti-TMEM119 antibody [28-3] - Microglial marker (ab209064)

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.

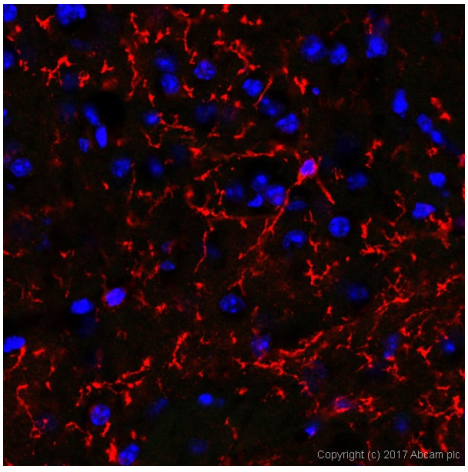
TMEM119 (green), Iba1 (red) and DAPI (blue)



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-TMEM119 antibody [28-3] - Microglial marker (ab209064)

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).

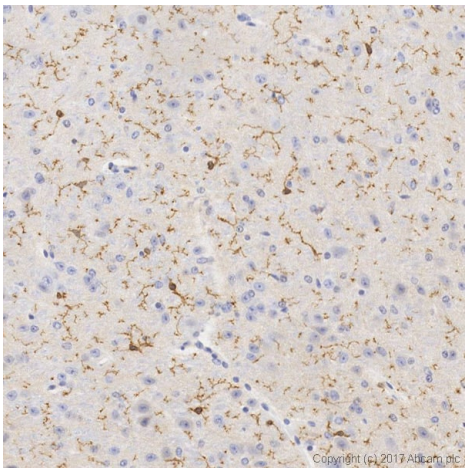
For other IHC staining systems (automated and non-automated), customers should optimize variable parameters such as antigen retrieval conditions, antibody concentrations and incubation times.



Immunohistochemistry (Frozen sections) - Anti-TMEM119 antibody [28-3] - Microglial marker (ab209064)

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).

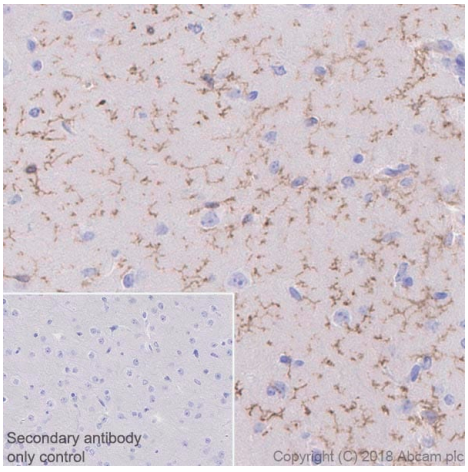
For other IHC staining systems (automated and non-automated), customers should optimize variable parameters such as antigen retrieval conditions, antibody concentrations and incubation times.



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-TMEM119 antibody [28-3] - Microglial marker (ab209064)

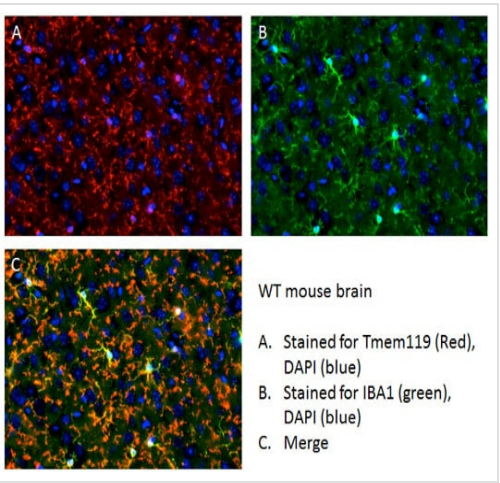
IHC image of TMEM119 staining in a section of formalin fixed, paraffin embedded normal mouse brain, performed on a Leica Bond™ system using the standard protocol B. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH6, epitope retrieval solution 1) for 20 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.



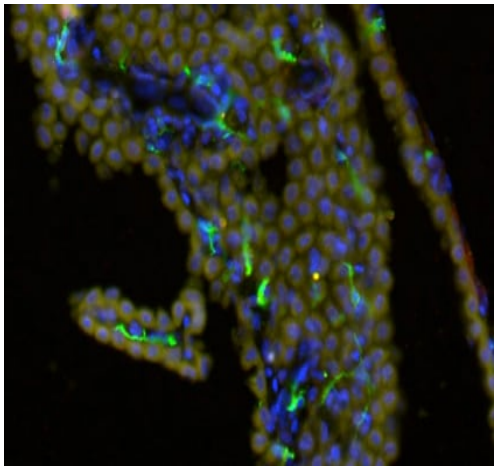
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-TMEM119 antibody [28-3] - Microglial marker (ab209064)

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.



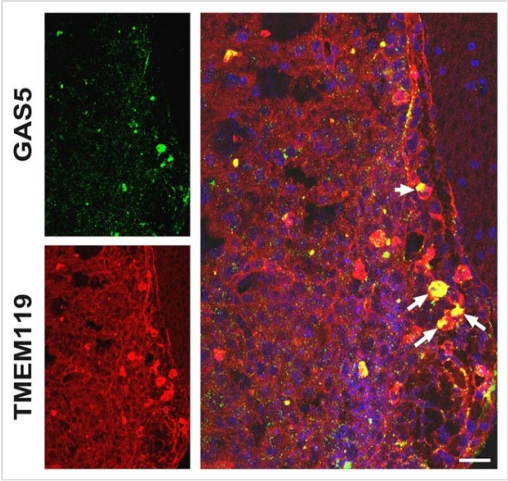
Immunohistochemistry (Frozen sections) - Anti-TMEM119 antibody [28-3] - Microglial marker (ab209064)

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 (Frozen sections) - Anti-TMEM119 antibody [28-3] - Microglial marker (ab209064)

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^{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.

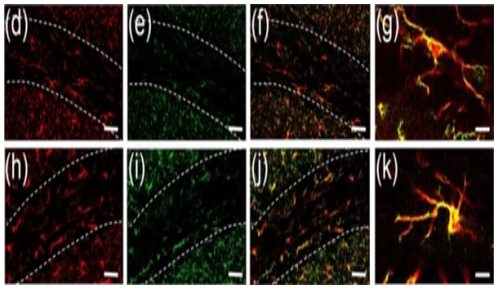


Immunohistochemistry (Frozen sections) - Anti-TMEM119 antibody [28-3] - Microglial marker (ab209064)

Representative FISH analysis of GAS5 (green) co**l**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.

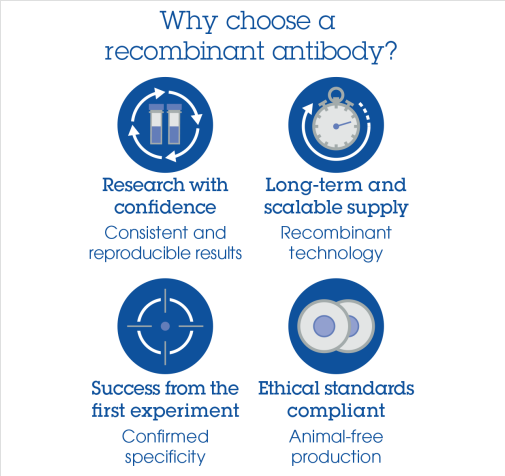
Tissue sections were fixed, permeabilized, and incubated with the primary antibody overnight at 4°C, followed by 2 h of incubation with TRITC**l** or FITC**l**conjugated secondary antibodies. Then, the samples were counterstained with Hoechst 33342.



Immunohistochemistry (Frozen sections) - Anti-TMEM119 antibody [28-3] - Microglial marker (ab209064)

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 $Iba\text{-}1^{+}$ 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.



Anti-TMEM119 antibody [28-3] - Microglial marker (ab209064)

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