# abcam

## Product datasheet

# Anti-GSDMD antibody [EPR20859] ab219800



ייבעדער RabMAb

★★★★★ 2 Abreviews 96 References 画像数9

### 製品の概要

製品名 Anti-GSDMD antibody [EPR20859]

製品の詳細 Rabbit monoclonal [EPR20859] to GSDMD

由来種 Rabbit

特異性 This antibody can detect full length, as well as a N-terminal fragment after stimulation in WB.

Expression level of GSDMD in whole normal brain lysate is low or undetectable (PMID:

32671214, PMID: 34975487), we recommend loading higher amount of lysate or using lower

antibody dilution.

アプリケーション 適用あり: Flow Cyt (Intra), IP, WB, IHC-P

適用なし: ICC/IF

種交差性 交差種: Mouse, Rat

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

ポジティブ・コントロール WB: Wild-type mouse liver and lung whole cell lysates; C6, PC-12, RAW 264.7 and NIH/3T3

whole cell lysates; Rat brain and liver lysates, IHC-P: Wild type mouse small intestine and mouse

lung tissues. Flow Cyt (intra): RAW 264.7 cells. IP: RAW 264.7 whole cell lysate.

特記事項 This product is a recombinant monoclonal antibody, which offers several advantages including:

- High batch-to-batch consistency and reproducibility

- Improved sensitivity and specificity

- Long-term security of supply

- Animal-free production

For more information see here.

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

保存方法 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.

バッファー pH: 7.2

Preservative: 0.01% Sodium azide

Constituents: PBS, 40% Glycerol, 0.05% BSA

精製度 Protein A purified

**ポリ/モノ** モノクローナル

**クローン名** EPR20859

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ab219800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。

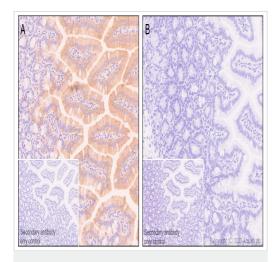
アプリケーション	Abreviews	特記事項
Flow Cyt (Intra)		1/500.
IP		1/30.
WB		1/1000 - 1/2000. Detects a band of approximately 53 kDa (predicted molecular weight: 53 kDa).
IHC-P	**** <u>(2)</u>	1/1000. Perform heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

追加情報 Is unsuitable for ICC/IF.

ターゲット情報

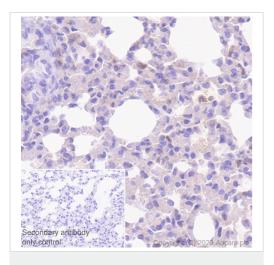
配列類似性 Belongs to the gasdermin family.

# 画像



Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-GSDMD antibody
[EPR20859] (ab219800)

Immunohistochemical analysis of paraffin-embedded (A) Small intestine tissue from wild-type C57BL/6JGpt mice (B) Small intestine tissue from GSDMD knockout mice staining with ab219800 at 1/5000 dilution and ready-to-use Goat Anti-Rabbit IgG H&L (HRP) secondary. Counterstaining with hematoxylin. Heat mediated antigen retrieval was performed with Citrate buffer (pH 6.0, Epitope Retrieval Solution 1) for 20 mins. Positive staining on (A) Small intestine tissue from wild-type C57BL/6JGpt mice and no staining on (B) Small intestine tissue from GSDMD knockout mice. The section was incubated with ab219800 for 30 mins at room temperature. The immunostaining was performed on a Leica Biosystems BOND™ RX instrument. The tissue samples were kindly provided by GemPharmatech. C57BL/6JGpt wildtype mice and GSDMD-KO homozygous mice (Strain ID: T010437).

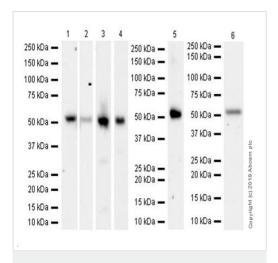


Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-GSDMD antibody
[EPR20859] (ab219800)

Immunohistochemical analysis of paraffin-embedded mouse lung tissue labelling GSDMD with ab219800 at 1/1000 (0.542 μg/ml) followed by <u>ab214880</u> at a ready to use dilution. **Low expression tissue:** weak staining on mouse lung. The section was incubated with ab219800 at 4°C overnight. Counterstained with hematoxylin. Secondary antibody only control: Secondary antibody is <u>ab214880</u>

at a ready to use dilution.

Heat mediated antigen retrieval was performed using <u>ab93684</u> (Tris/EDTA buffer, pH 9.0).



Western blot - Anti-GSDMD antibody [EPR20859] (ab219800)

**All lanes :** Anti-GSDMD antibody [EPR20859] (ab219800) at 1/1000 dilution

Lane 1: C6 (rat glial tumor cell line) whole cell lysate

**Lane 2**: PC-12 (rat adrenal gland pheochromocytoma cell line) whole cell lysate

Lane 3: RAW 264.7 (mouse macrophage cell line transformed

with Abelson murine leukemia virus) whole cell lysate

Lane 4: NIH/3T3 (mouse embyro fibroblast cell line) whole cell

ysate

Lane 5: Rat liver lysate

Lane 6: Rat brain lysate

Lysates/proteins at 10 µg per lane.

## **Secondary**

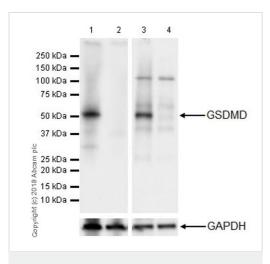
**All lanes :** Goat Anti-Rabbit lgG H&L (HRP) (<u>ab97051</u>) at 1/100000 dilution

Predicted band size: 53 kDa Observed band size: 53 kDa

**Exposure time :** Lanes 1 and 2:3 minutes; Lanes 3 and 4:48 seconds; Lanes 5 and 6:3 minutes.

Blocking/Dilution buffer: 5% NFDM/TBST.

Expression level of GSDMD in whole normal brain lysate is low or undetectable (PMID: 32671214, PMID: 34975487), we recommend loading higher amount of lysate or using lower antibody dilution.



Western blot - Anti-GSDMD antibody [EPR20859] (ab219800)

**All lanes :** Anti-GSDMD antibody [EPR20859] (ab219800) at 1/1000 dilution

Lane 1: Wild-type mouse liver whole cell lysate

Lane 2: GSDMD knockout mouse liver whole cell lysate

Lane 3: Wild-type mouse lung whole cell lysate

Lane 4: GSDMD knockout mouse lung whole cell 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

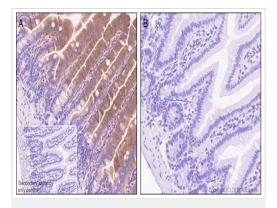
**Predicted band size:** 53 kDa **Observed band size:** 53 kDa

**Exposure time:** Lanes 1 and 2:15 seconds; Lanes 3 and 4:37 seconds.

Blocking/Dilution buffer: 5% NFDM/TBST.

The antibody has strong reactivity with GSDMD with a few weak bands of undermined proteins.

The lysates were kindly provided by our collaborator Dr Feng Shao's lab, NIBS.



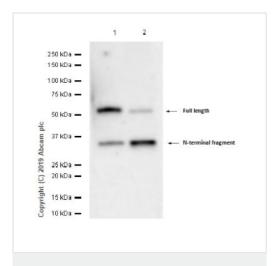
Immunohistochemistry (Formalin/PFA-fixed paraffinembedded sections) - Anti-GSDMD antibody
[EPR20859] (ab219800)

Immunohistochemical analysis of paraffin-embedded Wild type mouse small intestine (A) and GSDMD KO mouse small intestine (B) tissue labeling GSDMD with ab219800 at 1/1000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) Ready to use. Cytoplasmic staining on epithelium of wild type mouse small intestine (A), no staining on GSDMD KO mouse small intestine (B) (PMID: 17350798) is observed. Counter stained with hematoxylin.

Secondary antibody only control: Used PBS instead of primary antibody, secondary antibody is Goat Anti-Rabbit lgG H&L (HRP) Ready to use.

The tissue samples were kindly provided by Dr. Feng Shao NIBS.

Heat mediated antigen retrieval with Tris/EDTA buffer pH 9.0 was performed before commencing with IHC staining protocol.



Western blot - Anti-GSDMD antibody [EPR20859] (ab219800)

All lanes: Anti-GSDMD antibody [EPR20859] (ab219800)

Lane 1: iBMM (mouse immortalized bone marrow derived macrophages) treated with 500ng/ml Bsak plus 500ng/ml anthrax protective antigen (PA) for 2h. Cell lysate

Lane 2: iBMM (mouse immortalized bone marrow derived macrophages) treated with 500ng/ml Bsak plus 500ng/ml anthrax protective antigen (PA) for 2h. Concentrated cell supernatant

### **Secondary**

**All lanes :** Goat Anti-Rabbit IgG H&L (HRP) (<u>ab97051</u>) at 1/20000 dilution

Predicted band size: 53 kDa

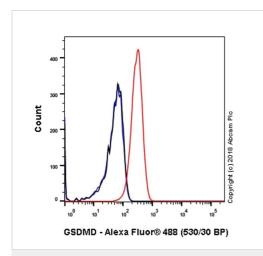
Observed band size: 31,53 kDa

Exposure time: 180 seconds

Blocking and diluting buffer: 5% NFDM /TBST

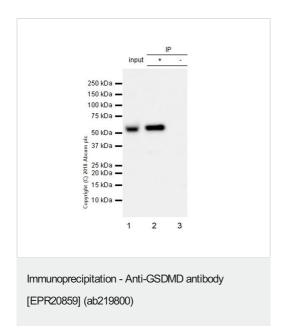
This antibody could detect full length, as well as a N-terminal fragment after stimulation.

The lysates were kindly provided by our Dr Feng Shao's lab, NIBS.



Flow Cytometry (Intracellular) - Anti-GSDMD antibody [EPR20859] (ab219800)

Intracellular flow cytometric analysis of 4% paraformaldehyde-fixed, 90% methanol-permeabilized RAW 264.7 (mouse macrophage cell line transformed with Abelson murine leukemia virus) cell line labeling GSDMD with ab219800 at 1/500 dilution (red) compared with a Rabbit lgG, monoclonal [EPR25A] - Isotype Control (ab172730) (black) and an unlabeled control (cells without incubation with primary antibody and secondary antibody) (blue). Goat Anti-Rabbit lgG H&L (Alexa Fluor® 488) (ab150077) at 1/2000 dilution was used as the secondary antibody.



GSDMD was immunoprecipitated from 0.35 mg of RAW 264.7 (mouse macrophage cell line transformed with Abelson murine leukemia virus) whole cell lysate with ab219800 at 1/30 dilution. Western blot was performed from the immunoprecipitate using ab219800 at 1/500 dilution. VeriBlot for IP Detection Reagent (HRP) (ab131366), was used for detection at 1/1000 dilution.

Lane 1: RAW 264.7 whole cell lysate 10 µg (Input).

Lane 2: ab219800 IP in RAW 264.7 whole cell lysate.

**Lane 3:** Rabbit monoclonal lgG (<u>ab172730</u>) instead of ab219800 in RAW 264.7 whole cell lysate.

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

Exposure time: 10 seconds.



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