# abcam

# Product datasheet

# Anti-GSDMD antibody [EPR19828] ab209845





★★★★★ 3 Abreviews 195 References 7 Images

#### Overview

**Product name** Anti-GSDMD antibody [EPR19828]

**Description** Rabbit monoclonal [EPR19828] to GSDMD

**Host species** Rabbit

Specificity In our hands this product works well in western blot using mouse cell lines but not tissue samples.

We recommend ab219800 as an alternative for low expression samples.

**Tested applications** Suitable for: WB, IP Species reactivity Reacts with: Mouse

**Immunogen** Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.

Positive control WB: RAW 264.7 cell line with ectopic expression of Apoptosis-associated speck-like protein,

> untreated and primed with 1  $\mu$ g/mL lipopolysaccharides for 4 h followed by 10  $\mu$ M nigericin treatment under serum starved conditions for 2 h, whole cell lysates; J774A.1. Mouse liver lysate IP: RAW 264.7-derived cells with ectopic expression of ASC, untreated and primed with 1 μg/mL lipopolysaccharides (LPS) for 4 h followed by 10 µM nigericin treatment under serum starved

conditions for 2 h, whole cell lysates.

**General notes** 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**.

### **Properties**

**Form** 

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: 59% PBS, 40% Glycerol, 0.05% BSA

Purity Protein A purified

Clone number Monoclonal EPR19828

**Isotype** IgG

# **Applications**

The Abpromise guarantee Our Abpromise guarantee covers the use of ab209845 in the following tested applications.

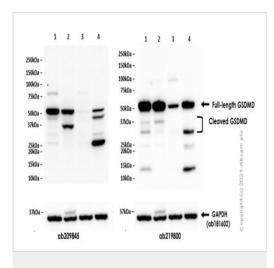
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
WB	**** <u>(2)</u>	1/1000. Detects a band of approximately 53, 32 kDa (predicted molecular weight: 53 kDa).
IP		1/30.

#### **Target**

**Sequence similarities** Belongs to the gasdermin family.

# **Images**



Western blot - Anti-GSDMD antibody [EPR19828] (ab209845)

**All lanes :** Anti-GSDMD antibody [EPR19828] (ab209845) at 1/1000 dilution (**ab219800**)

**Lane 1 :** RAW264.7 (mouse Abelson murine leukemia virus-induced tumor macrophage) whole cell lysate, Batch 1

Lane 2: Mouse liver lysate

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

lysate

**Lane 4 :** RAW264.7 (mouse Abelson murine leukemia virusinduced tumor macrophage) whole cell lysate, Batch 2

Lysates/proteins at 20 µg per lane.

## **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: 53 kDa Blocking buffer and concentration: 5% NFDM/TBST

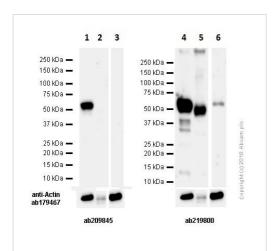
Diluting buffer and concentration: 5% NFDM/TBST

Exposure time: ab209845 - 120 seconds, ab219800 - 20 seconds

Compared with ab209845, <u>ab219800</u> has a higher affinity in positive samples.

The MW observed is consistent with the literature: PMID 26375003.

The 43 kDa band is likely to be a GSDMD cleavage product by caspase-3 at GSDMD Asp88 (PMID: 30902848, PMID: 28392147, PMID: 30135078).



Western blot - Anti-GSDMD antibody [EPR19828] (ab209845)

**Lanes 1-3**: Anti-GSDMD antibody [EPR19828] (ab209845) at 1/1000 dilution

**Lanes 4-6**: Anti-GSDMD antibody [EPR20859] (<u>ab219800</u>) at 1/1000 dilution

Lanes 1 & 4: Raw264.7 (Mouse Abelson murine leukemia virusinduced tumor macrophage) whole cell lysates

Lanes 2 & 5: Mouse liver tissue lysate

Lanes 3 & 6: Mouse spinal cord tissue lysate

Lysates/proteins at 20 µg per lane.

#### **Secondary**

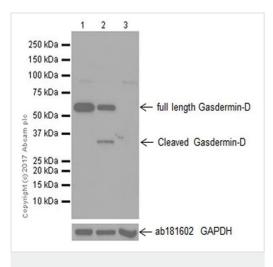
**All lanes :** Goat Anti-Rabbit lgG H&L (HRP) (ab97051) at 1/20000 dilution

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

Exposure time: 3 minutes

Blocking/Diluting buffer and concentration 5% NFDM/TBST.

Ab209845 work best with cell lysates.



Western blot - Anti-GSDMD antibody [EPR19828] (ab209845)

**All lanes :** Anti-GSDMD antibody [EPR19828] (ab209845) at 1/1000 dilution

**Lane 1**: RAW 264.7 (Mouse macrophage cell line transformed with Abelson murine leukemia virus)-derived cell line with ectopic expression of ASC (Apoptosis-associated speck-like protein), whole cell lysate

**Lane 2**: RAW 264.7 (Mouse macrophage cell line transformed with Abelson murine leukemia virus)-derived cell line with ectopic expression of ASC primed with 1  $\mu$ g/mL lipopolysaccharides (LPS) for 4 h followed by 10  $\mu$ M nigericin treatment under serum starved conditions for 2 h, whole cell lysate

**Lane 3**: Gasdermin-D knockout RAW 264.7 (Mouse macrophage cell line transformed with Abelson murine leukemia virus)-derived cell line with ectopic expression of ASC whole cell lysate

Lysates/proteins at 20 µg per lane.

#### Secondary

**All lanes :** Goat Anti-Rabbit IgG H&L (HRP) (<u>ab97051</u>) at 1/50000 dilution

Predicted band size: 53 kDa Observed band size: 32,53 kDa

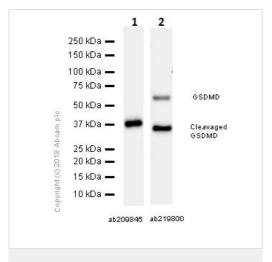
Exposure time: 1 minute

Blocking/Dilution buffer: 5% NFDM/TBST.

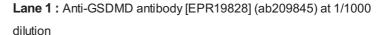
As a response to LPS stimulation and nigericin treatment,
Gasdermin-D is cleaved and Gasdermin-D N-terminal form is
detected at 32kDa. Details of RAW264.7-derived cells with ectopic
expression of ASC are described in the literature: PMID 26611636

The MW observed is consistent with the literature: PMID 26375003.

The cells were kindly provided by our collaborator Dr. Jiahuai Han, Xiamen University.



Western blot - Anti-GSDMD antibody [EPR19828] (ab209845)



Lane 2: Anti-GSDMD antibody [EPR20859] (ab219800) at 1/1000 dilution

**All lanes :** J774A.1 (Mouse reticulum cell sarcoma monocyte macrophage) whole cell lysates

Lysates/proteins at 20 µg per lane.

#### 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: 53 kDa

Exposure time: 3 minutes

Diluting/Blocking buffer and concentration 5% NFDM/TBST

1 2 3

250 kDa —
150 kDa —
100 kDa —
75 kDa —
50 kDa —
37 kDa —
37 kDa —

Cleaved
Gasdermin-D

Cleaved
Gasdermin-D

Cleaved
Gasdermin-D

Cleaved
Gasdermin-D

Immunoprecipitation - Anti-GSDMD antibody [EPR19828] (ab209845)

Gasdermin-D was immunoprecipitated from 0.35 mg of RAW 264.7 (Mouse macrophage cell line transformed with Abelson murine leukemia virus)-derived cells with ectopic expression of ASC were primed with 1 $\mu$ g/mL lipopolysaccharides (LPS) for 4 h followed by 10  $\mu$ M nigericin treatment under serum starved conditions for 2 h, whole cell lysate with ab209845 at 1/30 dilution.

Western blot was performed from the immunoprecipitate using ab209845 at 1/1000 dilution.

VeriBlot for IP Detection Reagent (HRP) (<u>ab131366</u>), was used for detection at 1/10000 dilution.

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

Exposure time: 3 minutes.

As a response to LPS stimulation and nigericin treatment,
Gasdermin-D is cleaved and Gasdermin-D N-terminal form is
detected at 32kDa. Details of RAW 264.7-derived cells with ectopic
expression of ASC are described in the literature: PMID 26611636.

The cells were kindly provided by our collaborator Dr. Jiahuai Han, Xiamen University.

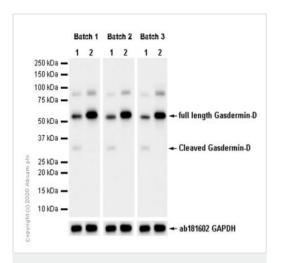
Note: The antibody has better affinity to full length Gasdermin-D.

#### All lanes:

**Lane 1 :** RAW 264.7-derived cells with ectopic expression of ASC were primed with 1 $\mu$ g/mL lipopolysaccharides (LPS) for 4 h followed by 10  $\mu$ M nigericin treatment under serum starved conditions for 2 h at 10  $\mu$ g

**Lane 2**: ab209845 IP in RAW 264.7-derived cells with ectopic expression of ASC were primed with  $1\mu g/mL$  lipopolysaccharides (LPS) for 4 h followed by 10  $\mu$ M nigericin treatment under serum starved conditions for 2 h whole cell lysate at 10  $\mu$ g

**Lane 3 :** Rabbit monoclonal IgG (<u>ab172730</u>) instead of ab209845 in RAW 264.7-derived cells with ectopic expression of ASC were primed with 1µg/mL lipopolysaccharides (LPS) for 4 h followed by 10 µM nigericin treatment under serum starved conditions for 2 h whole cell lysate



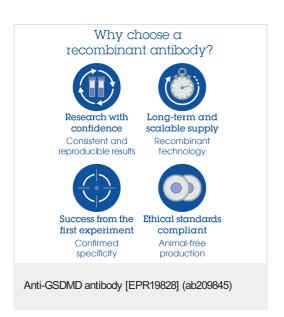
Western blot - Anti-GSDMD antibody [EPR19828] (ab209845)

Different batches of ab209845 were tested on:

1: RAW 264.7 (Mouse macrophage cell line transformed with Abelson murine leukemia virus) -derived cell line with ectopic expression of ASC primed with 1 µg/mL lipopolysaccharides (LPS) for 4 h followed by 10 uM nigericin treatment under serum starved conditions for 2 h, whole cell lysate at 5.2 µg/ml.

2: RAW 264.7 (Mouse macrophage cell line transformed with Abelson murine leukemia virus) -derived cell line with ectopic expression of ASC (Apoptosis-associated speck-like protein), whole cell lysate at 5.2 µg/ml.

 $15~\mu g$  of lysate was loaded in each lane. Bands observed at  $32{,}53~k Da$ .



Please note: All products are "FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES"

#### Our Abpromise to you: Quality guaranteed and expert technical support

- Replacement or refund for products not performing as stated on the datasheet
- Valid for 12 months from date of delivery
- Response to your inquiry within 24 hours
- We provide support in Chinese, English, French, German, Japanese and Spanish
- Extensive multi-media technical resources to help you
- We investigate all quality concerns to ensure our products perform to the highest standards

If the product does not perform as described on this datasheet, we will offer a refund or replacement. For full details of the Abpromise, please visit <a href="https://www.abcam.com/abpromise">https://www.abcam.com/abpromise</a> or contact our technical team.

#### Terms and conditions

• Guarantee only valid for products bought direct from Abcam or one of our authorized distributors