abcam

Product datasheet

Anti-GSDMD antibody [EPR19828] - BSA and Azide free ab225867

KO VALIDATED Recombinant RabMAb

1 References 5 Images

Overview

Product name	Anti-GSDMD antibody [EPR19828] - BSA and Azide free
Description	Rabbit monoclonal [EPR19828] to GSDMD - BSA and Azide free
Host species	Rabbit
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-derived cell line with ectopic expression of ASC (Apoptosis-associated speck-like protein), 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	ab225867 is the carrier-free version of **ab209845**. 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. 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	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	EPR19828
Isotype	IgG

Applications

The Abpromise guarantee Our **Abpromise guarantee** covers the use of ab225867 in the following tested applications.

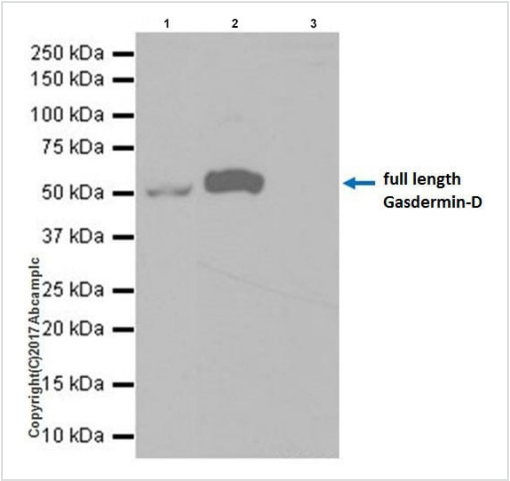
The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
WB		Use at an assay dependent concentration. Detects a band of approximately 53, 32 kDa (predicted molecular weight: 53 kDa).
IP		Use at an assay dependent concentration.

Target

Sequence similarities Belongs to the gasdermin family.

Images



Immunoprecipitation - Anti-GSDMD antibody [EPR19828] - BSA and Azide free (ab225867)

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 (Apoptosis-associated speck-like protein), 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) (**ab131366**), was used for detection at 1/10000 dilution.

Lane 1: RAW 264.7-derived cells with ectopic expression of ASC whole cell lysate 10ug (Input).

Lane 2: **ab209845** IP in RAW 264.7-derived cells with ectopic expression of ASC whole cell lysate.

Lane 3: Rabbit monoclonal IgG (**ab172730**) instead of **ab209845** in RAW 264.7-derived cells with ectopic expression of ASC whole cell

lysate.

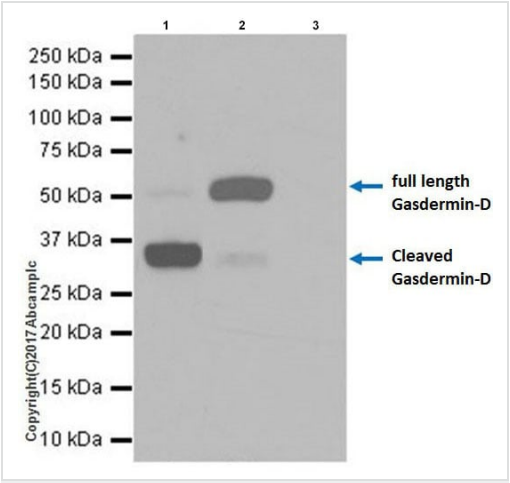
Blocking and dilution buffer and concentration: 5% NFDM/TBST.

Exposure time: 3 minutes.

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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab209845**).



Immunoprecipitation - Anti-GSDMD antibody [EPR19828] - BSA and Azide free (ab225867)

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 1µg/mL lipopolysaccharides (LPS) for 4 h followed by 10 µM 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) (**ab131366**), was used for detection at 1/10000 dilution.

Lane 1: 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 10ug (Input).

Lane 2: **ab209845** IP 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.

Lane 3: Rabbit monoclonal IgG (**ab172730**) 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.

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

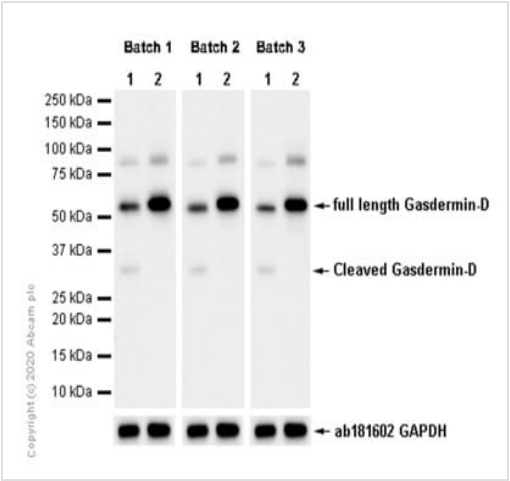
Exposure time: 3 minutes.

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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab209845**).



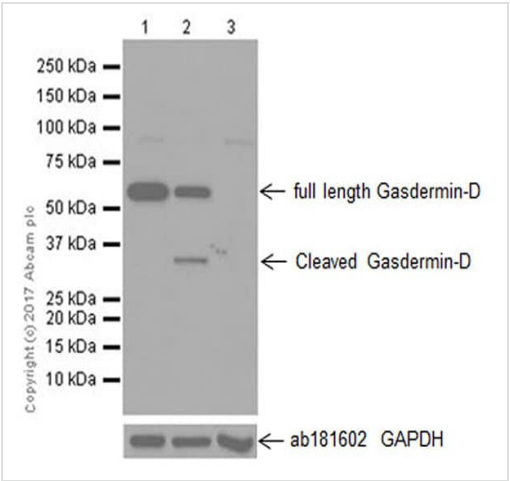
Western blot - Anti-GSDMD antibody [EPR19828] - BSA and Azide free (ab225867)

This data was developed using **ab209845**, the same antibody clone in a different buffer formulation. 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 µg of lysate was loaded in each lane. Bands observed at 32,53 kDa.



Western blot - Anti-GSDMD antibody [EPR19828] - BSA and Azide free (ab225867)

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 µg/mL lipopolysaccharides (LPS) for 4 h followed by 10 µM 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) (**ab97051**) at 1/50000

dilution

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

Exposure time: 1 minute

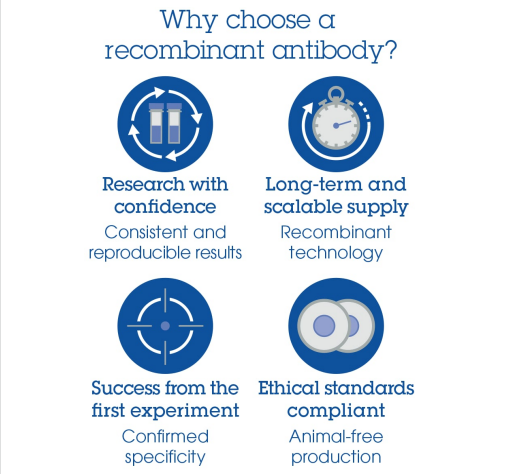
Blocking/Dilution buffer: 5% NFDM/TBST.

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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (**ab209845**).



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