abcam

ab239377

Anti-GSDMD antibody [EPR20859] - BSA and Azide free

Recombinant | RabMAb | KO Validated

Key facts

Isotype	IgG
Host species	Rabbit
Storage buffer	pH: 7.2 - 7.4 Constituents: PBS
Form	Liquid
Clonality	Monoclonal
Immunogen	The exact immunogen used to generate this antibody is proprietary information.
Carrier free	Yes
Clone number	EPR20859
Purification technique	Affinity purification Protein A
Specificity	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. Please see documentation for further information on positive controls (Chinese version)
Concentration	0.989 - 1.037 mg/mL The concentration of this product may be batch-dependent Batch concentration finder →

Reactivity data

IP

Tested

Species	Mouse
Dilution info	-
Notes	-

Expected

Species	Rat, Human
Dilution info	Use at an assay dependent concentration.
Notes	-

WB

Tested

Species	Mouse
Dilution info	-
Notes	-

Species	Rat
Dilution info	-
Notes	-

Species	Human
Dilution info	-
Notes	This antibody is only suitable to detect GSDMD in human cell lines. Not suitable for testing human tissues in WB.

Flow Cyt (Intra)

Tested

Species	Mouse
Dilution info	-
Notes	-

Expected

Species	Rat, Human
Dilution info	Use at an assay dependent concentration.
Notes	-

IHC-P

Tested

Species	Mouse
Dilution info	-
Notes	Perform heat-mediated antigen retrieval with Tris/EDTA buffer pH 9.0 before commencing with IHC staining protocol.

Not recommended

Species	Human, Rat
Dilution info	-
Notes	-

ICC/IF

Tested

Species	Mouse
Dilution info	-
Notes	-

Expected

Species	Rat, Human
Dilution info	Use at an assay dependent concentration.
Notes	-

Target data

See full target information Gsdmd

Function

Gasdermin-D. Precursor of a pore-forming protein that plays a key role in host defense against pathogen infection and danger signals (PubMed:26375003, PubMed:26375259, PubMed:26611636, PubMed:27383986, PubMed:27385778, PubMed:27418190). This form constitutes the precursor of the pore-forming protein: upon cleavage, the released N-terminal moiety (Gasdermin-D, N-terminal) binds to membranes and forms pores, triggering pyroptosis (PubMed:26375003, PubMed:26375259, PubMed:26611636, PubMed:27383986, PubMed:27385778, PubMed:27418190). Gasdermin-D, N-terminal. Promotes pyroptosis in response to microbial infection and danger signals (PubMed:26375003, PubMed:26375259, PubMed:26611636, PubMed:27383986, PubMed:27385778, PubMed:27418190, PubMed:32820063, PubMed:34289345, PubMed:35705808, PubMed:37988464, PubMed:38530158, PubMed:38538834, PubMed:38632402). Produced by the cleavage of gasdermin-D by inflammatory caspases CASP1 or CASP4/CASP11 in response to canonical, as well as non-canonical (such as cytosolic LPS) inflammasome activators (PubMed:26375003, PubMed:26375259, PubMed:26611636, PubMed:27383986, PubMed:27385778, PubMed:27418190, PubMed:35705808, PubMed:38632402). After cleavage, moves to the plasma membrane where it strongly binds to inner leaflet lipids, including monophosphorylated phosphatidylinositols, such as phosphatidylinositol 4-phosphate, bisphosphorylated phosphatidylinositols, such as phosphatidylinositol (4,5)-bisphosphate, as well as phosphatidylinositol (3,4,5)-bisphosphate, and more weakly to phosphatidic acid and phosphatidylserine (PubMed:27339137, PubMed:27383986). Homooligomerizes within the membrane and forms pores of 10-15 nanometers (nm) of inner diameter, allowing the release of mature interleukin-1 (IL1B and IL18) and triggering pyroptosis (PubMed:27383986, PubMed:29195811, PubMed:29274245, PubMed:33883744, PubMed:38530158, PubMed:38538834). Gasdermin pores also allow the release of mature caspase-7 (CASP7) (PubMed:35705808). In some, but not all, cells types, pyroptosis is followed by pyroptotic cell death, which is caused by downstream activation of ninjurin-1 (NINJ1), which mediates membrane rupture (cytolysis) (PubMed:38632402). Also forms pores in the mitochondrial membrane, resulting in release of mitochondrial DNA (mtDNA) into the cytosol (PubMed:37001519). Gasdermin-D, N-terminal released from pyroptotic cells into the extracellular milieu rapidly binds to and kills both Gram-negative and Gram-positive bacteria, without harming neighboring mammalian cells, as it does not disrupt the plasma membrane from the outside due to lipid-binding specificity (PubMed:27383986). Under cell culture conditions, also active against intracellular bacteria, such as Listeria monocytogenes (PubMed:27383986). Also active in response to MAP3K7/TAK1 inactivation by Yersinia toxin YopJ, which triggers cleavage by CASP8 and subsequent activation (PubMed:30361383, PubMed:30381458). Required for mucosal tissue defense against enteric pathogens (PubMed:37988464). Activation of the non-canonical inflammasome in brain endothelial cells can lead to excessive pyroptosis, leading to blood-brain barrier breakdown (PubMed:38632402). Strongly binds to bacterial and mitochondrial lipids, including cardiolipin. Does not bind to unphosphorylated phosphatidylinositol, phosphatidylethanolamine nor phosphatidylcholine (PubMed:27383986). Gasdermin-D, p13. Transcription coactivator produced by the cleavage by CASP3 or CASP7 in the upper small intestine in response to dietary antigens (PubMed:37327784). Required to maintain food tolerance in small intestine: translocates to the nucleus and acts as a coactivator for STAT1 to induce the transcription of CIITA and MHC class II molecules, which in turn induce type 1 regulatory T (Tr1) cells in upper small intestine (PubMed:37327784). Gasdermin-D, p40. Produced by the cleavage by

papain allergen (PubMed:35794369). After cleavage, moves to the plasma membrane and homooligomerizes within the membrane and forms pores of 10-15 nanometers (nm) of inner diameter, allowing the specific release of mature interleukin-33 (IL33), promoting type 2 inflammatory immune response (PubMed:35749514, PubMed:35794369).

Storage

Shipped at conditions	Blue Ice
Appropriate short-term storage conditions	+4°C
Appropriate long-term storage conditions	+4°C
Storage information	Do Not Freeze

Notes

ab239377 is the carrier-free version of ab219800.

Patented technology
Our RabMAb® technology is a patented hybridoma-based technology for making rabbit monoclonal antibodies. For details on our patents, please refer to RabMAb® patents.

What are the advantages of a recombinant monoclonal antibody?
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 batch production

For more information, read more on recombinant antibodies.

Conjugation ready
Our carrier-free antibodies are typically supplied in a PBS-only formulation, purified and free of BSA, sodium azide and glycerol. 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.

Compatibility
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.

Supplementary info

This supplementary information is collated from multiple sources and compiled automatically.

Activity summary	GSDMD also known as gasdermin D is a protein known for its role in pyroptosis a form of programmed cell death. Its molecular weight is approximately 53 kDa. Mechanically GSDMD operates by forming pores in cell membranes. These pores disrupt cellular homeostasis and eventually lead to cell lysis. GSDMD is mainly expressed in immune cells including macrophages and neutrophils. Researchers frequently use GSDMD Western blot and GSDMD ELISA for its detection and quantification in various studies.
Biological function summary	Gasdermin D functions in the execution of immune responses against infections. It acts as an effector molecule that participates directly in pyroptosis by disrupting mitochondrial membranes. GSDMD operates as part of a larger inflammasome complex initiated by inflammatory signals. The inflammasome activates inflammatory caspases that cleave GSDMD enabling its active form to execute pyroptosis. This process releases cytokines like IL-1β enhancing the inflammatory response.
Pathways	GSDMD is important in the pyroptosis pathway initiated by the inflammasome. This process involves Caspase-1 a protease responsible for cleaving pro-inflammatory cytokines and initiating pyroptosis. Another significant pathway includes NLRP3 inflammasome which acts upstream to activate Caspase-1 and subsequently GSDMD establishing the overall inflammatory response in the innate immune system. Through these pathways GSDMD interacts closely with proteins like IL-18 an essential inflammatory mediator.
Associated diseases and disorders	Gasdermin D has links to inflammatory diseases such as rheumatoid arthritis and sepsis. In rheumatoid arthritis the excessive activation of GSDMD leads to chronic joint inflammation mediated by activated immune cells. In sepsis over-activation of the pyroptosis pathway may cause severe systemic inflammation driven by GSDMD activity exacerbating cytokine release. Connections exist between GSDMD and other proteins such as Caspase-11 which can also initiate GSDMD cleavage independently and has roles in non-canonical inflammasome pathways influencing these conditions.

Product promise

Tested

We have tested this species and application combination and it works. It is covered by our product promise.

Expected

We have not tested this specific species and application combination in-house, but expect it will work. It is covered by our product promise.

Predicted

This species and application combination has not been tested, but we predict it will work based on strong homology. However, this combination is not covered by our product promise.

Not recommended

We do not recommend this combination. It is not covered by our product promise.

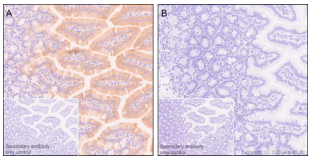
We are dedicated to supporting your work with high quality reagents and we are here for you every step of the way should you need us.

In the unlikely event of one of our products not working as expected, you are covered by our product promise.

Full details and terms and conditions can be found here:
Terms & Conditions.

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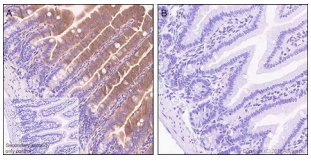
15 product images



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-GSDMD antibody [EPR20859] - BSA and Azide free (ab239377)

GSDMD Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-GSDMD antibody

This data was developed using ab219800, the same antibody clone in a different buffer formulation. 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 Tris-EDTA buffer (pH 9.0, Epitope Retrieval Solution2) 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 paraffin-embedded sections) - Anti-GSDMD antibody [EPR20859] - BSA and Azide free (ab239377)

GSDMD Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining using rabbit Anti-GSDMD antibody

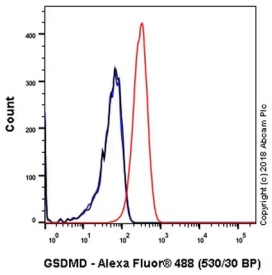
Immunohistochemical analysis of paraffin-embedded Wild type mouse small intestine (A) and GSDMD KO mouse small intestine (B) tissue labeling GSDMD with ab219800 at 1/1000 dilution, followed by Goat Anti-Rabbit IgG H&L (HRP) Ready to use. Cytoplasmic staining on epithelium of wild type mouse small intestine (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 IgG H&L (HRP) Ready to use.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab219800).

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

The tissue samples were kindly provided by Dr Feng Shao's lab, NIBS.

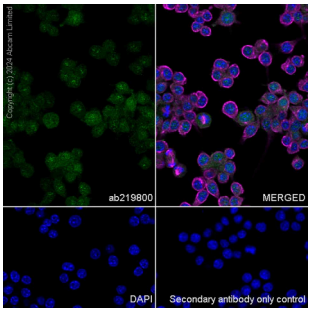


Flow Cytometry (Intracellular) - Anti-GSDMD antibody [EPR20859] - BSA and Azide free (ab239377)

GSDMD Flow Cytometry (Intracellular) staining of RAW 264.7 (mouse Abelson murine leukemia virus-induced tumor macrophage) cells using rabbit Anti-GSDMD antibody

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab219800).

Flow cytometry analysis of RAW 264.7 (mouse Abelson murine leukemia virus-induced tumor macrophage) cells stained with ab219800 (red) at 1/500 dilution (0.1 µg). Cells were fixed with 4% paraformaldehyde and permeabilized with 90% methanol. Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488, ab150077) was used as the secondary antibody at 1/2000 dilution. Rabbit monoclonal IgG (ab172730) was used as the isotype control (black). Cells without incubation with primary or secondary antibody were used as the unlabeled control (blue).



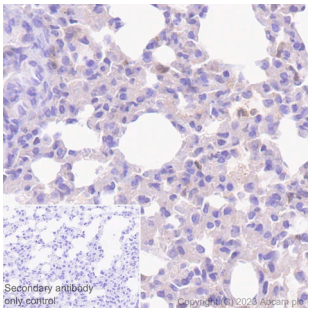
Immunocytochemistry/ Immunofluorescence - Anti-GSDMD antibody [EPR20859] - BSA and Azide free (ab239377)

GSDMD Immunocytochemistry/ Immunofluorescence staining of RAW 264.7 (mouse Abelson murine leukemia virus-induced tumor macrophage) using rabbit Anti-GSDMD antibody

This data was developed using ab219800, the same antibody clone in a different buffer formulation.
Immunofluorescent analysis of 4% Paraformaldehyde-fixed, 0.1% TritonX-100 permeabilized RAW 264.7 (mouse Abelson murine leukemia virus-induced tumor macrophage) cells labelling GSDMD with ab219800 at 1/50 dilution, followed by Goat Anti-Rabbit IgG H&L (Alexa Fluor® 488) preadsorbed (ab150081] secondary antibody at 1/1000 dilution.

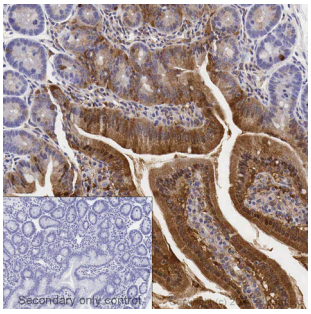
Confocal image showing cytoplasmic and nuclear staining in RAW 264.7 cell line (shown in green). The counterstain was observed in magenta. Nuclear DNA was labelled with DAPI (shown in blue). Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).

ab195889 Anti-alpha Tubulin mouse monoclonal antibody - Microtubule Marker (Alexa Fluor® 594) was used to counterstain tubulin at 1/200 (2.5 µg/ml) dilution (Magenta).



Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-GSDMD antibody [EPR20859] - BSA and Azide free (ab239377)

Immunohistochemical analysis of paraffin-embedded mouse lung tissue labelling GSDMD with ab219800 at 1/1000 (0.542 µg/ml) followed by ab214880 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 ab214880 at a ready to use dilution. Heat mediated antigen retrieval was performed using ab93684 (Tris/EDTA buffer, pH 9.0). This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab219800).



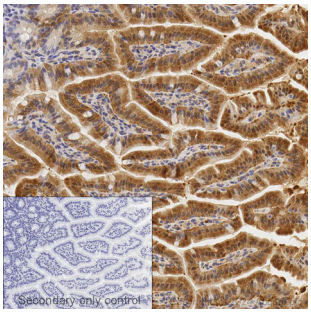
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-GSDMD antibody [EPR20859] - BSA and Azide free (ab239377)

GSDMD Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining of Mouse small intestine using rabbit Anti-GSDMD antibody

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab219800).
Immunohistochemical analysis of formalin fixed paraffin embedded mouse small intestine labelling GSDMD with ab219800 at a concentration of 0.05µ/ml. The immunostaining was performed on a Ventana DISCOVERY ULTRA (Roche Tissue Diagnostics) instrument with a OptiView DAB IHC Detection Kit. Heat mediated antigen retrieval was performed with DISCOVERY cell conditioning solution (CC1) 100°C, pH8.5 for 32mins.

ab219800 anti-GSDMD antibody [EPR20859] was incubated for 16mins at 37°C. Sections were counterstained with Hematoxylin II. Image inset shows absence of staining in secondary antibody only control.

Customers are encouraged to optimise antigen retrieval conditions, antibody concentration, incubation times and temperature for best results in their own IHC assay workflow (automated and manual)



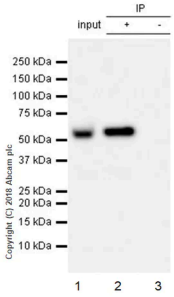
Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) - Anti-GSDMD antibody [EPR20859] - BSA and Azide free (ab239377)

GSDMD Immunohistochemistry (Formalin/PFA-fixed paraffin-embedded sections) staining of Mouse small intestine using rabbit Anti-GSDMD antibody

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab219800).
Immunohistochemical analysis of formalin fixed paraffin embedded mouse small intestine labelling GSDMD with ab219800 at a concentration of 0.1µ/ml. The immunostaining was performed on a Leica Biosystems BOND® RX instrument with a Bond™ Polymer Refine Detection kit. Heat mediated antigen retrieval was performed with Tris-EDTA buffer (pH 9.0, Epitope Retrieval Solution 2) for 20mins.

ab219800 anti-GSDMD antibody [EPR20859] was incubated for 15mins at room temperature. Sections were counterstained with Hematoxylin. Image inset shows absence of staining in secondary antibody only control.

Customers are encouraged to optimise antigen retrieval conditions, antibody concentration, incubation times and temperature for best results in their own IHC assay workflow (automated and manual)



Immunoprecipitation - Anti-GSDMD antibody [EPR20859] - BSA and Azide free (ab239377)

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 IgG (ab172730) instead of ab219800 in RAW 264.7 whole cell lysate.

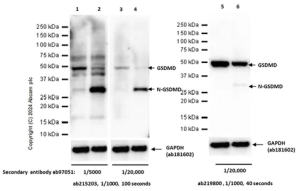
Blocking and dilution buffer and concentration: 5% NFDM/TBST.

Exposure time: 10 seconds.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab219800).

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

Predicted band size: 53 kDa



Western blot - Anti-GSDMD antibody [EPR20859] - BSA and Azide free (ab239377)

GSDMD Western blot staining using rabbit Anti-GSDMD antibody

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab219800).

ab215203 mainly recognizes N-terminal GSDMD and ab219800 mainly recognizes full length GSDMD.

Exposure time: Lanes 1-4: 100 seconds, Lanes 5-6: 40 seconds.

Lanes 1 - 4:
Western blot - Anti-cleaved N-terminal GSDMD antibody [EPR20829-408] (ab215203) at 1/1000 dilution

Lanes 5 - 6:
Western blot - Anti-GSDMD antibody [EPR20859] (ab219800) at 1/1000 dilution

Lanes 1, 3 and 5:
Untreated THP-1 (human epidermoid carcinoma cell line) whole cell lysate at 20 µg

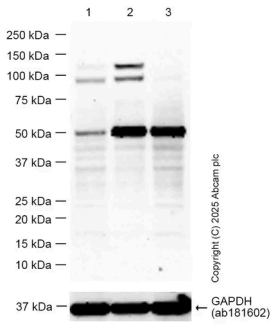
Lanes 2, 4 and 6:
THP-1 (human epidermoid carcinoma cell line) treated with 50ng/ml TPA for 24 hours, then treated with 5ng/ml LPS for 3 hours and add 1µg/ml BFA for another 3 h whole cell lysate at 20 µg

Secondary

Lanes 1, 2, 5 and 6:
Western blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/20000 dilution

Lanes 3 - 4:
Western blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/5000 dilution

Observed band size: 31 kDa



Western blot - Anti-GSDMD antibody [EPR20859] - BSA and Azide free (ab239377)

GSDMD Western blot staining using rabbit Anti-GSDMD antibody

This data was developed using ab219800, the same antibody clone in a different buffer formulation.

In Western blot, Anti-GSDMD antibody [EPR20859] (ab219800) 1:1,000 dilution (0.582 ug/ml) and Anti-GAPDH antibody [EPR16891] - Loading Control (ab181602) 1:1,000,000 dilution.

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

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

Lane 1:
HeLa (human cervix adenocarcinoma epithelial cell) whole cell lysate at 20 µg

Lane 2:
PC-3 (human prostate adenocarcinoma epithelial cell) whole cell lysate at 20 µg

Lane 3:
Jurkat (human T cell leukemia T lymphocyte) whole cell lysate at 20 µg

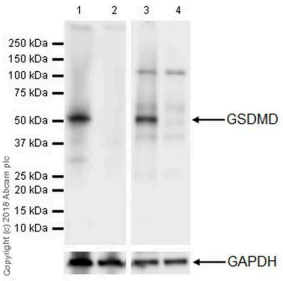
Secondary

All lanes:
Western blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/20000 dilution

Predicted band size: 53 kDa

Observed band size: 53 kDa, 37 kDa

Exposure time: 180s



Western blot - Anti-GSDMD antibody [EPR20859] - BSA and Azide free (ab239377)

GSDMD Western blot staining using rabbit Anti-GSDMD antibody

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.

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab219800).

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

Lane 1:
Wild-type mouse liver whole cell lysate at 20 µg

Lane 2:
GSDMD knockout mouse liver whole cell lysate at 20 µg

Lane 3:
Wild-type mouse lung whole cell lysate at 20 µg

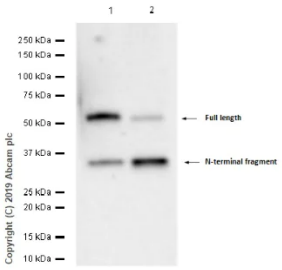
Lane 4:
GSDMD knockout mouse lung whole cell lysate at 20 µg

Secondary

All lanes:
Western blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/100000 dilution

Predicted band size: 53 kDa

Observed band size: 53 kDa



Western blot - Anti-GSDMD antibody [EPR20859] - BSA and Azide free (ab239377)

This data was developed using the same antibody clone in a different buffer formulation containing PBS, BSA, glycerol, and sodium azide (ab219800).

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 Dr Feng Shao's lab, NIBS.

All lanes:
Western blot - Anti-GSDMD antibody [EPR20859] (ab219800) at 1/2000 dilution

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

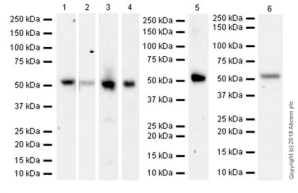
Lane 2:
iBMM (mouse immortalized bone marrow derived macrophages) treated with 500ng/ml Bsak plus 500ng/ml anthrax protective antigen (PA) for 2h. Concentrated cell supernatant

Secondary

All lanes:
Western blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/20000 dilution

Predicted band size: 53 kDa, 31 kDa

Observed band size: 31 kDa, 53 kDa



Western blot - Anti-GSDMD antibody [EPR20859] - BSA and Azide free (ab239377)

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.

This data was developed using ab219800, the same antibody clone in a different buffer formulation.
All lanes:
Western blot - Anti-GSDMD antibody [EPR20859] (ab219800) at 1/1000 dilution

Lane 1:
C6 (rat glial tumor cell line) whole cell lysate at 10 µg

Lane 2:
PC-12 (rat adrenal gland pheochromocytoma cell line) whole cell lysate at 10 µg

Lane 3:
RAW 264.7 (mouse macrophage cell line transformed with Abelson murine leukemia virus) whole cell lysate at 10 µg

Lane 4:
NIH/3T3 (mouse embyro fibroblast cell line) whole cell lysate at 10 µg

Lane 5:
Rat liver lysate at 10 µg

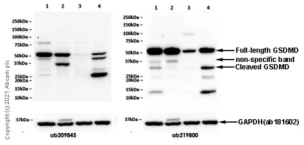
Lane 6:
Rat brain lysate at 10 µg

Secondary

All lanes:
Western blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/100000 dilution

Predicted band size: 53 kDa

Observed band size: 53 kDa



Western blot - Anti-GSDMD antibody [EPR20859] - BSA and Azide free (ab239377)

GSDMD Western blot staining using rabbit Anti-GSDMD antibody

This data was developed using ab219800, the same antibody clone in a different buffer formulation.
Compared with ab209845, ab219800 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).

We are unsure how to define the 37kDa extra band.

Other bands may be the GSDMD cleavage products, you can find them in PMID: 34890644, PMID: 33208231, PMID: 28679757.

Blocking and dilution buffer: 5% NFDM/TBST.

Lanes 1 - 4:
Western blot - Anti-GSDMD antibody [EPR19828] (ab209845) at 1/1000 dilution

Lanes 1 - 4:
Western blot - Anti-GSDMD antibody [EPR20859] (ab219800)

Lane 1:
RAW264.7 (mouse Abelson murine leukemia virus-induced tumor macrophage) whole cell lysate, Batch 1 at 20 µg

Lane 2:
Mouse liver lysate at 20 µg

Lane 3:
NIH/3T3 (mouse embyro fibroblast cell line) whole cell lysate at 20 µg

Lane 4:
RAW264.7 (mouse Abelson murine leukemia virus-induced tumor macrophage) whole cell lysate, Batch 2 at 20 µg

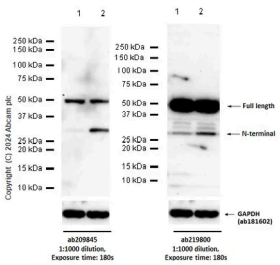
Secondary

All lanes:
Western blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/20000 dilution

Predicted band size: 53 kDa

Observed band size: 53 kDa

Exposure time: 120s



Western blot - Anti-GSDMD antibody [EPR20859] - BSA and Azide free (ab239377)

GSDMD Western blot staining using rabbit Anti-GSDMD antibody

This data was developed using ab219800, the same antibody clone in a different buffer formulation.
Blocking and dilution buffer: 5% NFDM/TBST.

Lanes 1 - 2:
Western blot - Anti-GSDMD antibody [EPR19828] (ab209845) at 1/1000 dilution

Lanes 1 - 2:
Western blot - Anti-GSDMD antibody [EPR20859] (ab219800)

Lane 1:
J774A.1 (Mouse reticulum cell sarcoma monocyte macrophage) whole cell lysate at 20 µg

Lane 2:
J774A.1 (Mouse reticulum cell sarcoma monocyte macrophage) treated with 100ng/ml lipopolysaccharides (LPS) for 3h, then added 300ng/ml BFA for another 3h whole cell lysate at 20 µg

Secondary

All lanes:
Western blot - Goat Anti-Rabbit IgG H&L (HRP) (ab97051) at 1/20000 dilution

Performed under reducing conditions.

Predicted band size: 53 kDa

Observed band size: 53 kDa

Exposure time: 180s

Please note: All products are 'FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC OR THERAPEUTIC PROCEDURES'.