Anti-Gasdermin D (N terminal) Antibody [PD00-18] - BSA and Azide free

HA750486

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IHC-P, IF-Cell
Molecular Wt:	Predicted band size: 53/30 kDa
Clone number:	PD00-18

Description:	Gasdermin-D, N-terminal: Promotes pyroptosis in response to microbial infection and danger signals. Produced by the cleavage of gasdermin-D by inflammatory caspases CASP1 or CASP4 in response to canonical, as well as non-canonical (such as cytosolic LPS) inflammasome activators. 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. Homooligomerizes within the membrane and forms pores of 10 - 15 nanometers (nm) of inner diameter, possibly allowing the release of mature IL1B and triggering pyroptosis . Exhibits bactericidal activity. 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.
Immunogen:	Recombinant protein within Gasdermin D full length protein.
Positive control:	SiHa cell lysate, Jurkat cell lysate, THP-1 cell lysate, PC-3M cell lysate, NIH/3T3 cell lysate, PC-12 cell lysate, L6 cell lysate, THP-1 treated with 100nM TPA overnight then add 100ng/mL LPS for 7 hours then add 1μg/mL BFA for 3 hours cell lysate, mouse intestine tissue, L6.
Subcellular location:	Cell membrane, Secreted.
Database links:	SwissProt: P57764 Human | Q9D8T2 Mouse Entrez Gene: 513939 Rat
Recommended Dilutions:	
WB	1:2,000-1:5,000
IHC-P	1:1,000
IF-Cell	1:100
Storage Buffer:	PBS (pH7.4).
Storage Instruction:	Store at +4℃ after thawing. Aliquot store at -20℃. Avoid repeated freeze / thaw cycles.
Purity:	Protein A affinity purified.

Images

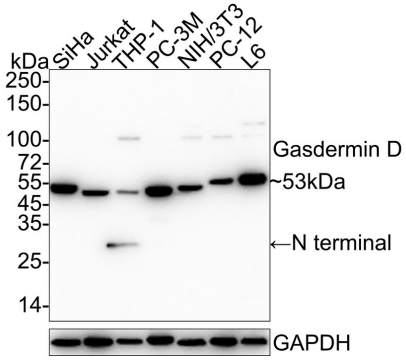

Fig1: Western blot analysis of Gasdermin D (N terminal) on different lysates with Rabbit anti-Gasdermin D (N terminal) antibody (HA750486) at 1/2,000 dilution.

Lane 1: SiHa cell lysate
Lane 2: Jurkat cell lysate
Lane 3: THP-1 cell lysate
Lane 4: PC-3M cell lysate
Lane 5: NIH/3T3 cell lysate
Lane 6: PC-12 cell lysate
Lane 7: L6 cell lysate

Lysates/proteins at 20 µg/Lane.

Predicted band size: 53/30 kDa
Observed band size: 53/30 kDa

Exposure time: 43 seconds; ECL: K1801;
4-20% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (HA750486) at 1/2,000 dilution was used in 5% NFDM/TBST at 4℃ overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

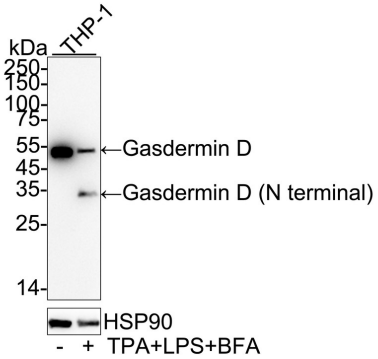

Fig2: Western blot analysis of Gasdermin D (N terminal) on different lysates with Rabbit anti-Gasdermin D (N terminal) antibody (HA750486) at 1/2,000 dilution.

Lane 1: THP-1 cell lysate (20 µg/Lane)
Lane 2: THP-1 treated with 100nM TPA overnight then add 100ng/mL LPS for 7 hours then add 1µg/mL BFA for 3 hours cell lysate (20 µg/Lane)

Predicted band size: 53/30 kDa
Observed band size: 53/35 kDa

Exposure time: 2 minutes; ECL: K1801;
4-20% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (HA750486) at 1/2,000 dilution was used in 5% NFDM/TBST at 4℃ overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

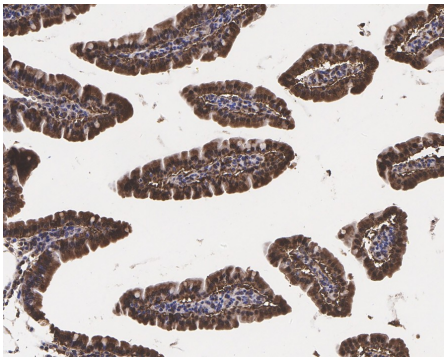

Fig3: Immunohistochemical analysis of paraffin-embedded mouse intestine tissue with Rabbit anti-Gasdermin D (N terminal) antibody (HA750486) at 1/1,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (HA750486) at 1/1,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

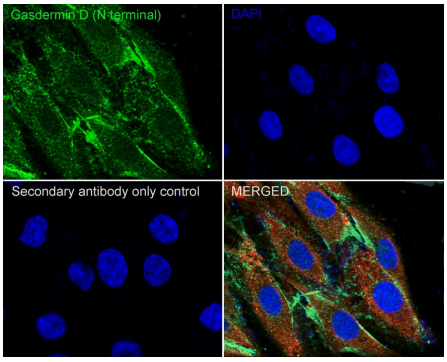

Fig4: Immunocytochemistry analysis of L6 cells labeling Gasdermin D (N terminal) with Rabbit anti-Gasdermin D (N terminal) antibody (HA750486) at 1/100 dilution.

Cells were fixed in 4% paraformaldehyde for 15 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 15 minutes at room temperature, then blocked with 1% BSA in 10% negative goat serum for 1 hour at room temperature. Cells were then incubated with Rabbit anti-Gasdermin D (N terminal) antibody (HA750486) at 1/100 dilution in 1% BSA in PBST overnight at 4 ℃. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. PBS instead of the primary antibody was used as the secondary antibody only control. Nuclear DNA was labelled in blue with DAPI.

Beta tubulin (HA601187, red) was stained at 1/100 dilution overnight at +4℃. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

Background References

1. Sborgi L. et. al. GSDMD membrane pore formation constitutes the mechanism of pyroptotic cell death. EMBO J. 35:1766-1778(2016).
2. Ding J. et. al. Pore-forming activity and structural autoinhibition of the gasdermin family. Nature 535:111-116(2016).