10320 Camino Santa Fe, Suite G
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Anti-GSDMD Antibody Picoband™ (monoclonal, 6D11)

Catalog # ABO15066

Specification

Anti-GSDMD Antibody Picoband™ (monoclonal, 6D11) - Product Information

Application	**WB, IHC, IF, ICC, FC**
Primary Accession	P57764
Host	**Mouse**
Isotype	**Mouse IgG2b**
Reactivity	**Human**
Clonality	**Monoclonal**
Format	**Lyophilized**

Description
Anti-GSDMD Antibody Picoband™ (monoclonal, 6D11) . Tested in Flow Cytometry, IF, IHC, ICC, WB applications. This antibody reacts with Human.

Reconstitution
Adding 0.2 ml of distilled water will yield a concentration of 500 µg/ml.

Anti-GSDMD Antibody Picoband™ (monoclonal, 6D11) - Additional Information

Gene ID 79792

Other Names
Gasdermin-D, Gasdermin domain-containing protein 1, Gasdermin-D, N-terminal, GSDMD-NT, Gasdermin-D, 13 kDa, 13 kDa GSDMD, Gasdermin-D, p40, GSDMD {ECO:0000303|PubMed:26375003, ECO:0000312|HGNC:HGNC:25697}

Calculated MW
53 kDa KDa

Application Details
Western blot, 0.25-0.5 µg/ml, Human
 Immunohistochemistry(Paraffin-embedded Section), 2-5 µg/ml, Human
 Immunocytochemistry/Immunofluorescence, 5 µg/ml, Human
 Flow Cytometry, 1-3 µg/1x10^6 cells, Human

Contents
Each vial contains 4 mg Trehalose, 0.9 mg NaCl and 0.2 mg Na2HPO4.

Immunogen
E.coli-derived human GSDMD recombinant protein (Position: M1-H484).

Purification
Immunogen affinity purified.

Storage	**At -20°C for one year from date of receipt. After reconstitution, at 4°C for one month. It can also be aliquotted and stored frozen at -20°C for six months. Avoid repeated**

freezing and thawing.

Anti-GSDMD Antibody Picoband™ (monoclonal, 6D11) - Protein Information

Name GSDMD {ECO:0000303|PubMed:26375003, ECO:0000312|HGNC:HGNC:25697}

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:27281216). 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:27281216).

Cellular Location
[Gasdermin-D]: Cytoplasm, cytosol. Inflammasome {ECO:0000250|UniProtKB:Q9D8T2}. Note=In response to a canonical inflammasome stimulus, such as nigericin, recruited to NLRP3 inflammasone with similar kinetics to that of uncleaved CASP1 precursor. {ECO:0000250|UniProtKB:Q9D8T2} [Gasdermin-D, N-terminal]: Cytoplasm, cytosol. Note=(Microbial infection) Upon infection by M.tuberculosis, localization to cell membrane is prevented by M.tuberculosis phosphatase PtpB that catalyzes dephosphorylation of phosphatidylinositol (4,5)-bisphosphate and phosphatidylinositol 4- phosphate, thereby inhibiting the membrane targeting of Gasdermin-D, N- terminal and subsequent cytokine release and pyroptosis [Gasdermin-D, C-terminal]: Cytoplasm, cytosol {ECO:0000250|UniProtKB:Q9D8T2}

Tissue Location
Expressed in the suprabasal cells of esophagus, as well as in the isthmus/neck, pit, and gland of the stomach, suggesting preferential expression in differentiating cells

Anti-GSDMD Antibody Picoband™ (monoclonal, 6D11) - Protocols

Provided below are standard protocols that you may find useful for product applications.

- Western Blot
- Blocking Peptides
- Dot Blot
- Immunohistochemistry
- Immunofluorescence
- Immunoprecipitation
- Flow Cytomety
- Cell Culture

Anti-GSDMD Antibody Picoband™ (monoclonal, 6D11) - Images

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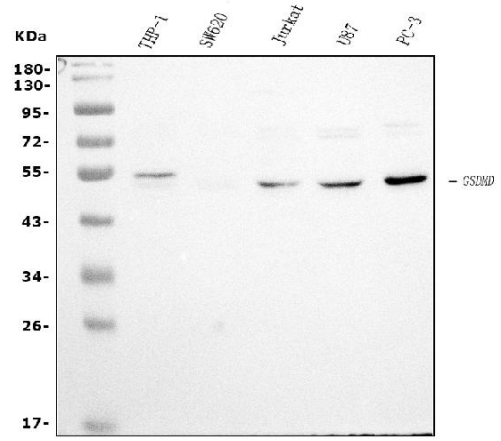

Figure 1. Western blot analysis of GSDMD using anti-GSDMD antibody (M02842).
Electrophoresis was performed on a 5-20% SDS-PAGE gel at 70V (Stacking gel) / 90V (Resolving gel) for 2-3 hours. The sample well of each lane was loaded with 30 ug of sample under reducing conditions.
Lane 1: human THP-1 whole cell lysates,
Lane 2: human SW620 whole cell lysates,
Lane 3: human Jurkat whole cell lysates,
Lane 4: human U87 whole cell lysates,
Lane 5: human PC-3 whole cell lysates.
After electrophoresis, proteins were transferred to a nitrocellulose membrane at 150 mA for 50-90 minutes. Blocked the membrane with 5% non-fat milk/TBS for 1.5 hour at RT. The membrane was incubated with mouse anti-GSDMD antigen affinity purified monoclonal antibody (Catalog # M02842) at 0.5 µg/mL overnight at 4°C, then washed with TBS-0.1%Tween 3 times with 5 minutes each and probed with a goat anti-mouse IgG-HRP secondary antibody at a dilution of 1:10000 for 1.5 hour at RT. The signal is developed using an Enhanced Chemiluminescent detection (ECL) kit (Catalog # EK1001) with Tanon 5200 system. A specific band was detected for GSDMD at approximately 53 kDa. The expected band size for GSDMD is at 53 kDa.

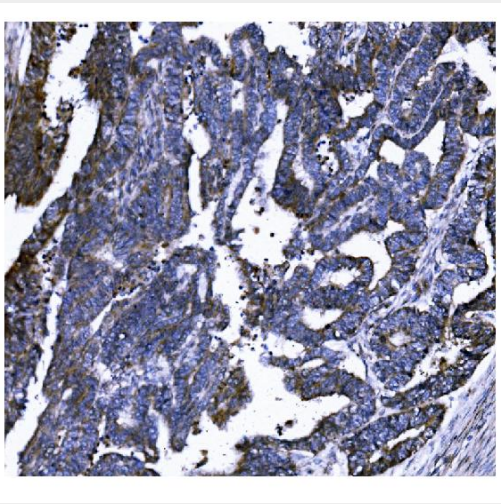

Figure 2. IHC analysis of GSDMD using anti-GSDMD antibody (M02842).
GSDMD was detected in a paraffin-embedded section of human cervical cancern tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH 8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 µg/ml mouse anti-GSDMD Antibody (M02842) overnight at 4°C. Biotinylated goat anti-mouse IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was

developed using Strepavidin-Biotin-Complex (SABC) (Catalog # SA1021) with DAB as the chromogen.

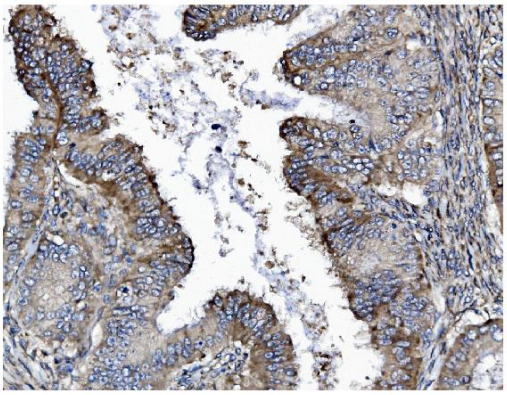

Figure 3. IHC analysis of GSDMD using anti-GSDMD antibody (M02842).
GSDMD was detected in a paraffin-embedded section of human cervical intraepithelial neoplasia tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH 8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 µg/ml mouse anti-GSDMD Antibody (M02842) overnight at 4°C. Biotinylated goat anti-mouse IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Strepavidin-Biotin-Complex (SABC) (Catalog # SA1021) with DAB as the chromogen.

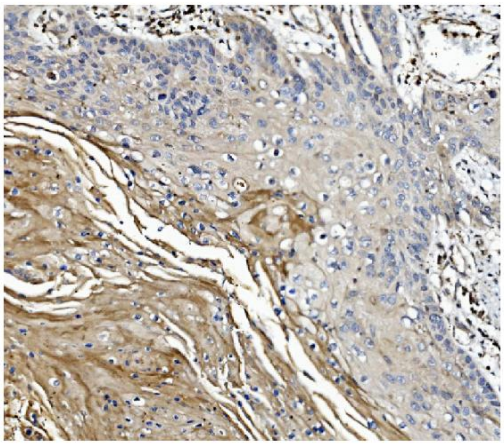

Figure 4. IHC analysis of GSDMD using anti-GSDMD antibody (M02842).
GSDMD was detected in a paraffin-embedded section of human esophageal squamous carcinoma tissue. Heat mediated antigen retrieval was performed in EDTA buffer (pH 8.0, epitope retrieval solution). The tissue section was blocked with 10% goat serum. The tissue section was then incubated with 2 µg/ml mouse anti-GSDMD Antibody (M02842) overnight at 4°C. Biotinylated goat anti-mouse IgG was used as secondary antibody and incubated for 30 minutes at 37°C. The tissue section was developed using Strepavidin-Biotin-Complex (SABC) (Catalog # SA1021) with DAB as the chromogen.

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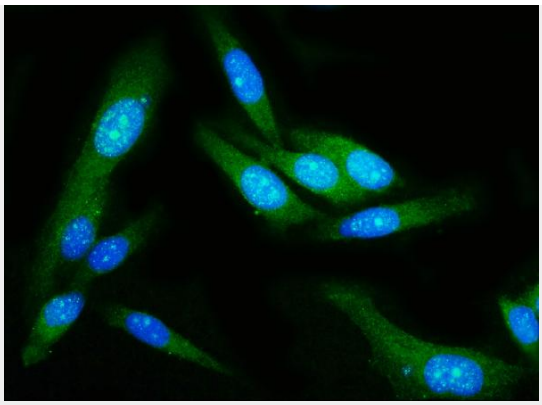

Figure 5. IF analysis of GSDMD using anti-GSDMD antibody (M02842).
GSDMD was detected in an immunocytochemical section of PC-3 cells. Enzyme antigen retrieval was performed using IHC enzyme antigen retrieval reagent (AR0022) for 15 mins. The cells were blocked with 10% goat serum. And then incubated with 5 µg/mL mouse anti-GSDMD Antibody (M02842) overnight at 4°C. DyLight®488 Conjugated Goat Anti-Mouse IgG (BA1126) was used as secondary antibody at 1:100 dilution and incubated for 30 minutes at 37°C. The section was counterstained with DAPI. Visualize using a fluorescence microscope and filter sets appropriate for the label used.

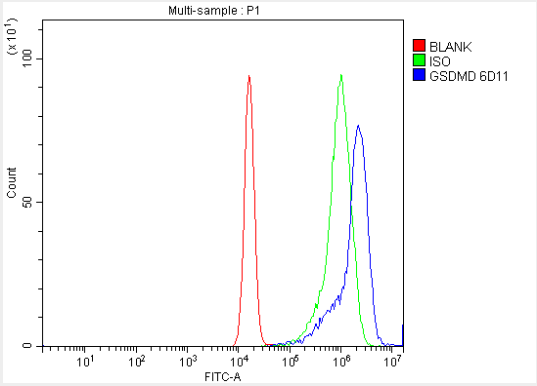

Figure 6. Flow Cytometry analysis of Jurkat cells using anti-GSDMD antibody (M02842).
Overlay histogram showing Jurkat cells stained with M02842 (Blue line). The cells were blocked with 10% normal goat serum. And then incubated with mouse anti-GSDMD Antibody (M02842, 1 µg/1x10^6 cells) for 30 min at 20°C. DyLight®488 conjugated goat anti-mouse IgG (BA1126, 5-10 µg/1x10^6 cells) was used as secondary antibody for 30 minutes at 20°C. Isotype control antibody (Green line) was mouse IgG (1 µg/1x10^6) used under the same conditions. Unlabelled sample (Red line) was also used as a control.

Anti-GSDMD Antibody Picoband™ (monoclonal, 6D11) - Background

Gasdermin D is a member of the gasdermin family. Members of this family appear to play a role in regulation of epithelial proliferation. Gasdermin D has been suggested to act as a tumor suppressor. Alternatively spliced transcript variants have been described.