Anti-DFNA5 / GSDME Antibody

ER1901-12



Product Type: Rabbit polyclonal IgG, primary antibodies

Species reactivity: Human

Applications: WB, IHC-P, FC

Molecular Wt: Predicted band size: 55 kDa

Description: Plays a role in the TP53-regulated cellular response to DNA damage probably by

cooperating with TP53. Gasdermin-E, N-terminal: Switches CASP3-mediated apoptosis induced by TNF or danger signals, such as chemotherapy drugs, to pyroptosis. Produced by the cleavage of GSDME by CASP3, perforates cell membrane and thereby induces pyroptosis. After cleavage, moves to the plasma membrane where it strongly binds to inner leaflet lipids, bisphosphorylated phosphatidylinositols, such as phosphatidylinositol (4,5)-bisphosphate. Mediates secondary necrosis downstream of the mitochondrial apoptotic pathway and CASP3 activation as well as in response to viral agents. Exhibits bactericidal

activity.

Immunogen: Recombinant protein within Human DFNA5 / GSDME aa 1-220 / 496.

Positive control: Hela cell lysates, SiHa cell lysates, rat testis tissue, human thyroid gland tissue, human

breast tissue, mouse small intestine tissue.

Subcellular location: Cell membrane, Cytoplasm, cytosol.

Database links: SwissProt: 060443 Human

Recommended Dilutions:

WB 1:500-1:1,000 IHC-P 1:50-1:200 FC 1:50-1:100

Storage Buffer: 1*PBS (pH7.4), 0.2% BSA, 50% Glycerol. Preservative: 0.05% Sodium Azide.

Storage Instruction: Store at $+4^{\circ}$ C after thawing. Aliquot store at -20° C. Avoid repeated freeze / thaw cycles.

Purity: Immunogen affinity purified.

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Images

170-130-100-70-55-40-35-25**Fig1:** Western blot analysis of DFNA5 / GSDME on different lysates with Rabbit anti-DFNA5 / GSDME antibody (ER1901-12) at 1/500 dilution.

Lane 1: Hela cell lysate Lane 2: Siha cell lysate

Lysates/proteins at 10 µg/Lane.

Predicted band size: 55 kDa Observed band size: 55 kDa

Exposure time: 2 minutes;

10% 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 (ER1901-12) at 1/500 dilution was used in 5% NFDM/TBST at room temperature for 2 hours. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1:300,000 dilution was used for 1 hour at room temperature.

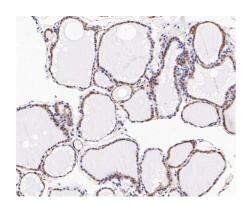


Fig2: Immunohistochemical analysis of paraffin-embedded human thyroid gland tissue using anti-DFNA5 / GSDME antibody. The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 8.0-8.4) for 20 minutes. The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH₂O and PBS, and then probed with the primary antibody (ER1901-12, 1/200) for 30 minutes 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.

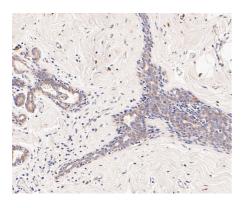


Fig3: Immunohistochemical analysis of paraffin-embedded human breast tissue using anti-DFNA5 / GSDME antibody. The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 8.0-8.4) for 20 minutes. The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH₂O and PBS, and then probed with the primary antibody (ER1901-12, 1/200) for 30 minutes 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.

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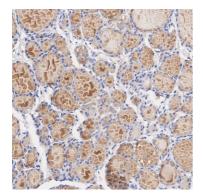


Fig4: Immunohistochemical analysis of paraffin-embedded rat thyroid tissue with Rabbit anti-DFNA5 / GSDME antibody (ER1901-12) at 1/200 dilution.

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 ddH₂O and PBS, and then probed with the primary antibody (ER1901-12) at 1/200 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.

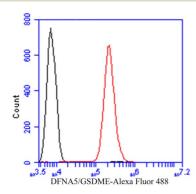


Fig5: Flow cytometric analysis of DFNA5 / GSDME was done on SiHa cells. The cells were fixed, permeabilized and stained with the primary antibody (ER1901-12, 1/50) (red). After incubation of the primary antibody at room temperature for an hour, the cells were stained with a Alexa Fluor 488-conjugated Goat anti-Rabbit IgG Secondary antibody at 1/1000 dilution for 30 minutes.Unlabelled sample was used as a control (cells without incubation with primary antibody; black).

Note: All products are "FOR RESEARCH USE ONLY AND ARE NOT INTENDED FOR DIAGNOSTIC OR THERAPEUTIC USE".

Background References

- 1. Masuda Y et al. The potential role of DFNA5, a hearing impairment gene, in p53-mediated cellular response to DNA damage. J. Hum. Genet. 51:652-664 (2006).
- 2. Rogers C et al. Cleavage of DFNA5 by caspase-3 during apoptosis mediates progression to secondary necrotic/pyroptotic cell death. Nat. Commun. 8:14128-14128 (2017).