Anti-STING Antibody [JM03-47]

ET1705-68

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Rat
Applications:	WB, IHC-P, IF-Cell, FC, IF-Tissue, IP
Molecular Wt:	Predicted band size: 42 kDa
Clone number:	JM03-47

Description: Facilitator of innate immune signaling that promotes the production of type I interferon (IFN-alpha and IFN-beta). Innate immune response is triggered in response to non-CpG double-stranded DNA from viruses and bacteria delivered to the cytoplasm. Able to activate both NF-kappa-B and IRF3 transcription pathways to induce expression of type I interferon and exert a potent anti-viral state following expression. May be involved in translocon function, the translocon possibly being able to influence the induction of type I interferons. May be involved in transduction of apoptotic signals via its association with the major histocompatibility complex class II (MHC-II). Mediates death signaling via activation of the extracellular signal-regulated kinase (ERK) pathway.

Immunogen: Recombinant protein within human STING aa 117-379.

Positive control: THP-1 cell lysate, SW620 cell lysate, human lung tissue lysate, THP-1, human tonsil tissue, rat lung tissue.

Subcellular location: Endoplasmic reticulum membrane, Cytoplasm, perinuclear region, Endoplasmic reticulum-Golgi intermediate compartment membrane, Golgi apparatus membrane, Cytoplasmic vesicle, autophagosome membrane, Mitochondrion outer membrane, Cell membrane.

Database links: SwissProt: Q86WV6 Human | F1M391 Rat

Recommended Dilutions:

WB	1:1,000
IHC-P	1:1,000
IF-Cell	1:100
FC	1:1,000
IF-Tissue	1:200
IP	1-2μg/sample

Storage Buffer: 1*TBS (pH7.4), 0.05% BSA, 40% Glycerol. Preservative: 0.05% Sodium Azide.

Storage Instruction: Shipped at 4℃. Store at +4℃ short term (1-2 weeks). It is recommended to aliquot into single-use upon delivery. Store at -20℃ long term.

Purity: Protein A affinity purified.

Images

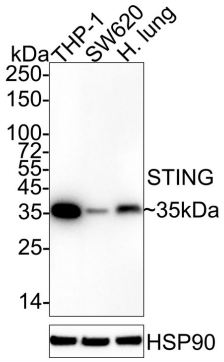

Fig1: Western blot analysis of STING on different lysates with Rabbit anti-STING antibody (ET1705-68) at 1/1,000 dilution.

Lane 1: THP-1 cell lysate (15 µg/Lane)
Lane 2: SW620 cell lysate (15 µg/Lane)
Lane 3: Human lung tissue lysate (30 µg/Lane)

Predicted band size: 42 kDa
Observed band size: 35 kDa

Exposure time: 1 minute 2 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 (ET1705-68) at 1/1,000 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.

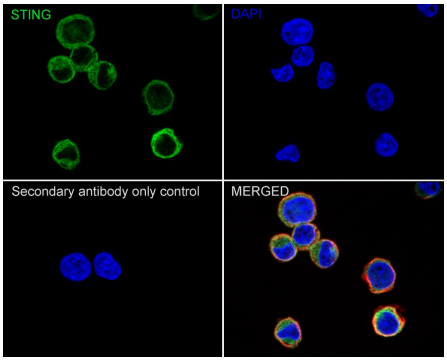

Fig2: Immunocytochemistry analysis of THP-1 cells labeling STING with Rabbit anti-STING antibody (ET1705-68) at 1/100 dilution.

Cells were fixed in 4% paraformaldehyde for 20 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 5 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-STING antibody (ET1705-68) 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 (M1305-2, 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.

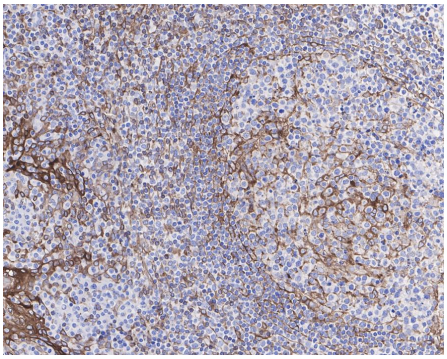

Fig3: Immunohistochemical analysis of paraffin-embedded human tonsil tissue with Rabbit anti-STING antibody (ET1705-68) at 1/1,000 dilution.

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 (ET1705-68) 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.

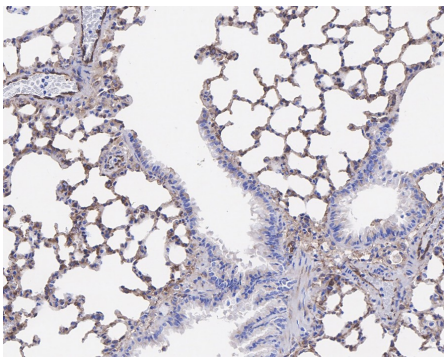

Fig4: Immunohistochemical analysis of paraffin-embedded rat lung tissue with Rabbit anti-STING antibody (ET1705-68) at 1/1,000 dilution.

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 (ET1705-68) 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.

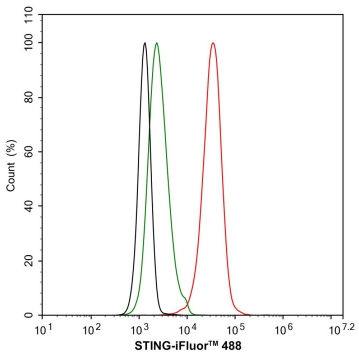

Fig5: Flow cytometric analysis of THP-1 cells labeling STING.

Cells were fixed and permeabilized. Then stained with the primary antibody (ET1705-68, 1/1,000) (red) compared with Rabbit IgG Isotype Control (green). After incubation of the primary antibody at +4℃ for an hour, the cells were stained with a iFluor™ 488 conjugate-Goat anti-Rabbit IgG Secondary antibody (HA1121) at 1/1,000 dilution for 30 minutes at +4℃. Unlabelled sample was used as a control (cells without incubation with primary antibody; black).

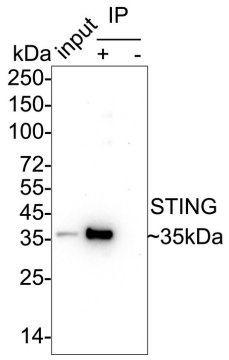

Fig6: STING was immunoprecipitated from 0.2 mg THP-1 cell lysate with ET1705-68 at 2 µg/10 µl beads. Western blot was performed from the immunoprecipitate using ET1705-68 at 1/1,000 dilution. Anti-Rabbit IgG for IP Nano-secondary antibody (NBI01H) at 1/5,000 dilution was used for 1 hour at room temperature.

Lane 1: THP-1 cell lysate (input)
Lane 2: ET1705-68 IP in THP-1 cell lysate
Lane 3: Rabbit IgG instead of ET1705-68 in THP-1 cell lysate

Blocking/Dilution buffer: 5% NFDM/TBST
Exposure time: 3 minutes; ECL: K1801

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

Background References

1. Panchanathan R et al. Identification of a negative feedback loop between cyclic di-GMP-induced levels of IFI16 and p202 cytosolic DNA sensors and STING. Innate Immun 20(7):751-9 (2014).
2. Orzalli MH et al. Nuclear interferon-inducible protein 16 promotes silencing of herpesviral and transfected DNA. Proc Natl Acad Sci U S A 110:E4492-501 (2013).