Anti-Fragilis Antibody [JU73-02]

ET1706-09

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human
Applications:	WB, IF-Cell, IF-Tissue, IHC-P, FC
Molecular Wt:	Predicted band size: 15 kDa
Clone number:	JU73-02

Description: Interferon-induced transmembrane protein 3 (IFITM3) is a protein that in humans is encoded by the IFITM3 gene. It plays a critical role in the immune system's defense against Swine Flu, where heightened levels of IFITM3 keep viral levels low, and the removal of IFITM3 allows the virus to multiply unchecked. This observation has been further advanced by a recent study from Paul Kellam's lab that shows that a single nucleotide polymorphism in the human IFITM3 gene purported to increase influenza susceptibility is overrepresented in people hospitalised with pandemic H1N1. The prevalence of this mutation is thought to be approximately 1/400 in European populations.

Immunogen: Synthetic peptide within Human Fragilis aa 1-50 / 133.

Positive control: HepG2 cell lysate, HeLa cell lysate, THP-1 cell lysate, HeLa, human breast cancer tissue, human colon cancer tissue, MCF-7.

Subcellular location: Cell membrane, Late endosome membrane, Lysosome membrane, perinuclear region.

Database links: SwissProt: Q01628 Human

Recommended Dilutions:

WB	1:2,000
IF-Cell	1:100
IF-Tissue	1:50-1:200
IHC-P	1:1,000
FC	1:50-1:100

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

Storage Instruction: Store at +4℃ after thawing. Aliquot store at -20℃ or -80℃. Avoid repeated freeze / thaw cycles.

Purity: Protein A affinity purified.

Images

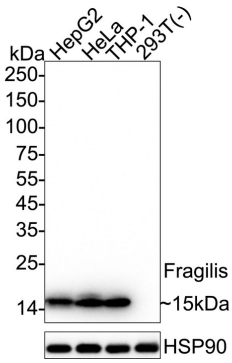

Fig1: Western blot analysis of Fragilis on different lysates with Rabbit anti-Fragilis antibody (ET1706-09) at 1/2,000 dilution.

Lane 1: HepG2 cell lysate
Lane 2: HeLa cell lysate
Lane 3: THP-1 cell lysate
Lane 4: 293T cell lysate (negative)

Lysates/proteins at 15 µg/Lane.

Predicted band size: 15 kDa
Observed band size: 15 kDa

Exposure time: 4 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 (ET1706-09) 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.

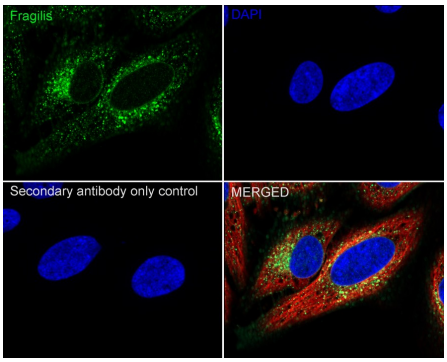

Fig2: Immunocytochemistry analysis of HeLa cells labeling Fragilis with Rabbit anti-Fragilis antibody (ET1706-09) at 1/100 dilution.

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-Fragilis antibody (ET1706-09) 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.

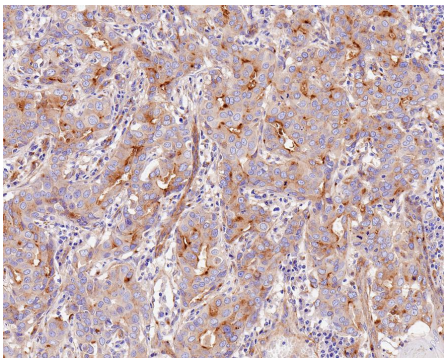

Fig3: Immunohistochemical analysis of paraffin-embedded human breast cancer tissue with Rabbit anti-Fragilis antibody (ET1706-09) at 1/200 dilution.

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 ddH_2O and PBS, and then probed with the primary antibody (ET1706-09) 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.

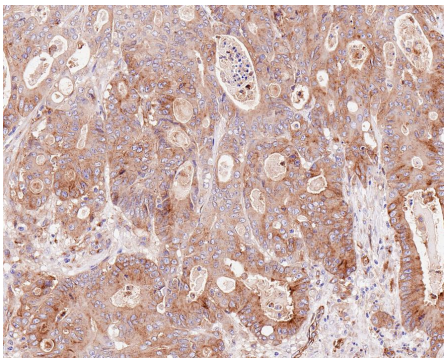

Fig4: Immunohistochemical analysis of paraffin-embedded human colon cancer tissue with Rabbit anti-Fragilis antibody (ET1706-09) at 1/200 dilution.

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 ddH_2O and PBS, and then probed with the primary antibody (ET1706-09) 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.

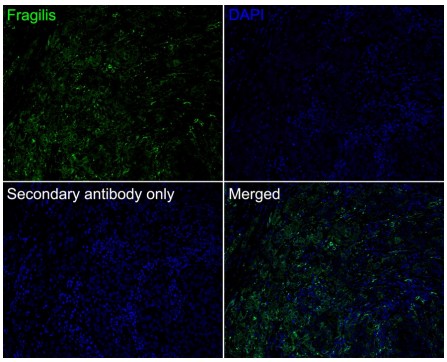

Fig5: Immunofluorescence analysis of paraffin-embedded human breast cancer tissue labeling Fragilis with Rabbit anti-Fragilis antibody (ET1706-09) at 1/200 dilution.

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 10% negative goat serum for 1 hour at room temperature, washed with PBS, and then probed with the primary antibody (ET1706-09, green) at 1/200 dilution overnight at 4 ℃, washed with PBS. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. Nuclei were counterstained with DAPI (blue).

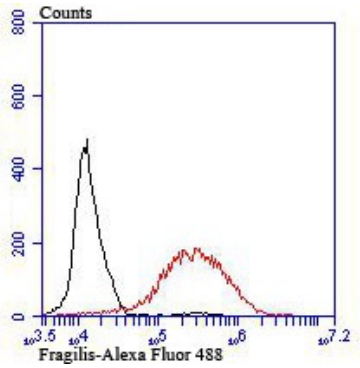

Fig6: Flow cytometric analysis of Fragilis was done on MCF-7 cells. The cells were fixed, permeabilized and stained with the primary antibody (ET1706-09, 1/50) (red). After incubation of the primary antibody at room temperature for an hour, the cells were stained with a Alexa Fluor 488-conjugated Goat 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).

Background References

1. Matsukawa K et al. Familial Amyotrophic Lateral Sclerosis-linked Mutations in Profilin 1 Exacerbate TDP-43-induced Degeneration in the Retina of Drosophila melanogaster through an Increase in the Cytoplasmic Localization of TDP-43. J Biol Chem 291:23464-23476 (2016).
2. Guo Z et al. A Legionella effector modulates host cytoskeletal structure by inhibiting actin polymerization. Microbes Infect N/A:N/A (2013).