Anti-Fatty Acid Synthase Antibody [JJ0939]

ET1701-91

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IF-Cell, IF-Tissue, IHC-P, IP, FC
Molecular Wt:	Predicted band size: 273 kDa
Clone number:	JJ0939

Description: Fatty acid biosynthesis is mediated by seven catalytic enzymes and an acyl carrier protein (ACP), to which various acyl intermediates are covalently attached. Fatty Acid Synthase (FAS) is the anabolic enzyme that contains the seven unique catalytic sites and mediates the conversion of acetyl-CoA and malonyl-CoA, in the presence of the cofactor NADPH, into long-chain saturated fatty acids, such as palmitate. Human Fatty Acid Synthase cDNA encodes a 2,504 amino acid protein. Catalytically active Fatty Acid Synthase is a homodimer. Human Fatty Acid Synthase mRNA is variably expressed with abundant levels present in brain, lung and liver. Fatty acid synthetic metabolism is abnormally elevated in tumor cells and may support cell growth or survival of malignant cancers.

Immunogen: Synthetic peptide within C-terminal human Fatty Acid Synthase.

Positive control: HeLa, HeLa cell lysate, HEK-293 cell lysate, A549 cell lysate, C2C12 cell lysate, L-929 cell lysate, L6 cell lysate, Mouse white adipose tissue lysate, Rat white adipose tissue lysate, Rat brain tissue lysate, C2C12, L6, SK-Br-3, human breast carcinoma tissue, mouse colon tissue, human liver tissue.

Subcellular location: Cytoplasm, Melanosome.

Database links: SwissProt: P49327 Human | P19096 Mouse | P12785 Rat

Recommended Dilutions:

WB	1:5,000
IF-Cell	1:100-1:500
IF-Tissue	1:100-1:500
IHC-P	1:50-1:200
FC	1:1,000

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

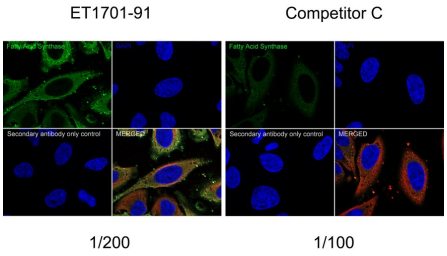

Fig1: Immunocytochemistry analysis of HeLa cells labeling Fatty Acid Synthase with Rabbit anti-Fatty Acid Synthase antibody (ET1701-91) at 1/200 dilution and competitor's antibody at 1/100 dilution.

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-Fatty Acid Synthase antibody (ET1701-91) at 1/200 dilution and competitor's antibody 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.

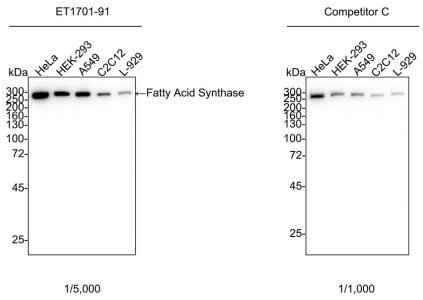

Fig2: Western blot analysis of Fatty Acid Synthase on different lysates with Rabbit anti-Fatty Acid Synthase antibody (ET1701-91) at 1/5,000 dilution and competitor's antibody at 1/1,000 dilution.

Lane 1: HeLa cell lysate
Lane 2: HEK-293 cell lysate
Lane 3: A549 cell lysate
Lane 4: C2C12 cell lysate
Lane 5: L-929 cell lysate

Lysates/proteins at 20 µg/Lane.

Predicted band size: 273 kDa
Observed band size: 273 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 (ET1701-91) at 1/5,000 dilution and competitor's antibody at 1/1,000 dilution were 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.

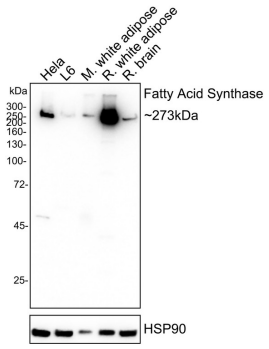

Fig3: Western blot analysis of Fatty Acid Synthase on different lysates with Rabbit anti-Fatty Acid Synthase antibody (ET1701-91) at 1/1,000 dilution.

Lane 1: HeLa cell lysate
Lane 2: L6 cell lysate
Lane 3: Mouse white adipose tissue lysate
Lane 4: Rat white adipose tissue lysate
Lane 5: Rat brain tissue lysate

Cell lysates/proteins at 20 µg/Lane.
Tissue lysates/proteins at 40 µg/Lane.

Predicted band size: 273 kDa
Observed band size: 273 kDa

Exposure time: 10 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 (ET1701-91) 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.

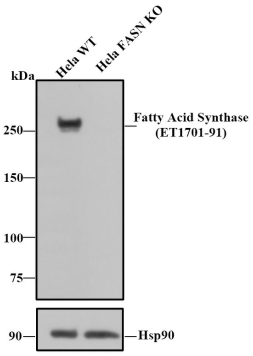

Fig4: All lanes: Western blot analysis of Fatty Acid Synthase with anti-Fatty Acid Synthase antibody [JJ0939] (ET1701-91) at 1:1,000 dilution.

Lane 1: Wild-type Hela whole cell lysate.
Lane 2: FASN knockout Hela whole cell lysate.

ET1701-91 was shown to specifically react with Fatty Acid Synthase in wild-type Hela cells. No band was observed when FASN knockout samples were tested. Wild-type and FASN knockout samples were subjected to SDS-PAGE. Proteins were transferred to a PVDF membrane and blocked with 5% NFDM in TBST for 1 hour at room temperature. The primary Anti-Fatty Acid Synthase antibody (ET1701-91, 1/1,000) and Anti-HSP90 antibody (ET1605-56, 1/10,000) were used in 5% BSA at room temperature for 2 hours. Goat Anti-Rabbit IgG H&L (HRP) Secondary Antibody (HA1001) at 1:200,000 dilution was used for 1 hour at room temperature.

Cell lysate was provided by Ubigene Biosciences (Ubigene Biosciences Co., Ltd., Guangzhou, China).

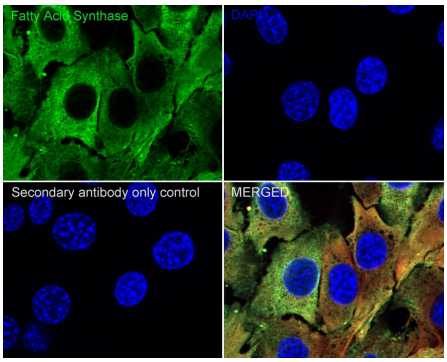

Fig5: Immunocytochemistry analysis of C2C12 cells labeling Fatty Acid Synthase with Rabbit anti-Fatty Acid Synthase antibody (ET1701-91) at 1/100 dilution.

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-Fatty Acid Synthase antibody (ET1701-91) 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.

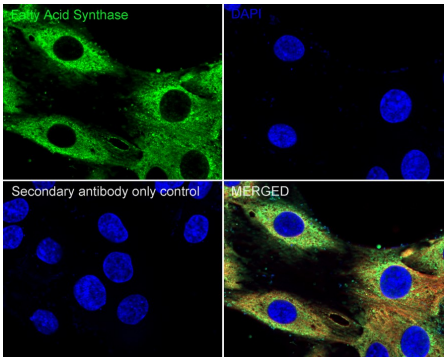

Fig6: Immunocytochemistry analysis of L6 cells labeling Fatty Acid Synthase with Rabbit anti-Fatty Acid Synthase antibody (ET1701-91) at 1/100 dilution.

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-Fatty Acid Synthase antibody (ET1701-91) 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.

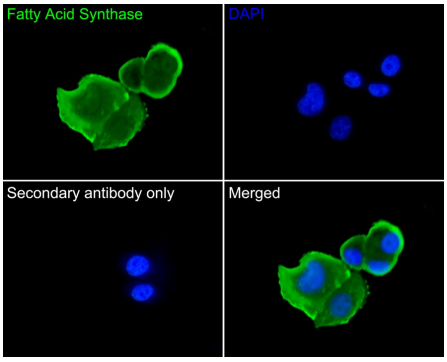

Fig7: Immunocytochemistry analysis of SK-Br-3 cells labeling Fatty Acid Synthase with Rabbit anti-Fatty Acid Synthase antibody (ET1701-91) at 1/50 dilution.

Cells were fixed in 4% paraformaldehyde for 10 minutes at 37 ℃, permeabilized with 0.05% Triton X-100 in PBS for 20 minutes, and then blocked with 2% negative goat serum for 30 minutes at room temperature. Cells were then incubated with Rabbit anti-Fatty Acid Synthase antibody (ET1701-91) at 1/50 dilution in 2% negative goat serum 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.

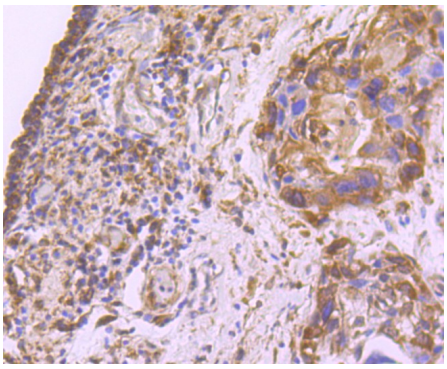

Fig8: Immunohistochemical analysis of paraffin-embedded human breast carcinoma tissue using anti-Fatty Acid Synthase antibody. The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 8.0-8.4) for 20 minutes.The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1701-91, 1/50) 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.

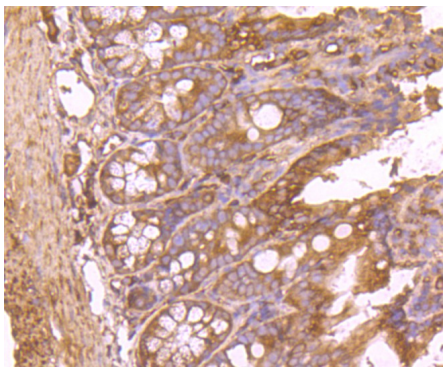

Fig9: Immunohistochemical analysis of paraffin-embedded mouse colon tissue using anti-Fatty Acid Synthase antibody. The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 8.0-8.4) for 20 minutes.The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1701-91, 1/50) 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.

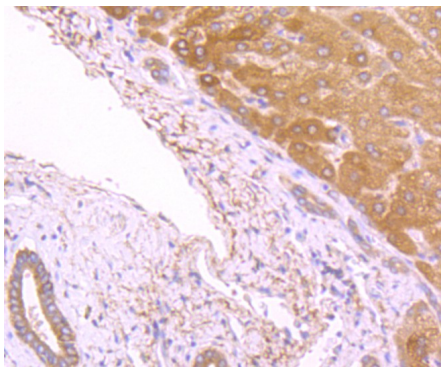

Fig10: Immunohistochemical analysis of paraffin-embedded human liver tissue using anti-Fatty Acid Synthase antibody. The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 8.0-8.4) for 20 minutes.The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1701-91, 1/50) 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.

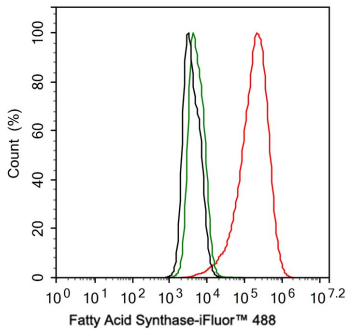

Fig11: Flow cytometric analysis of HeLa cells labeling Fatty Acid Synthase.

Cells were fixed and permeabilized. Then stained with the primary antibody (ET1701-91, 1μg/mL) (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).

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

Background References

1. Rolyan H et al. Defects of Lipid Synthesis Are Linked to the Age-Dependent Demyelination Caused by Lamin B1 Overexpression. J Neurosci 35:12002-17 (2015).
2. Li J et al. Fatty acid synthase mediates the epithelial-mesenchymal transition of breast cancer cells. Int J Biol Sci 10:171-80 (2014).