Anti-LFNG Antibody [JE66-62]

HA721367

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse
Applications:	WB, IF-Cell, FC
Molecular Wt:	Predicted band size: 42 kDa
Clone number:	JE66-62

Description: Glycosyltransferase that initiates the elongation of O-linked fucose residues attached to EGF-like repeats in the extracellular domain of Notch molecules. Modulates NOTCH1 activity by modifying O-fucose residues at specific EGF-like domains resulting in inhibition of NOTCH1 activation by JAG1 and enhancement of NOTCH1 activation by DLL1 via an increase in its binding to DLL1 (By similarity).

Immunogen: Synthetic peptide within Human LFNG aa 330-379 / 379.

Positive control: Hela cell lysate, K-562 cell lysate, HL-60 cell lysate, MCF7 cell lysate, HT-29 cell lysate, C2C12 cell lysate, PC-12 cell lysate, mouse testis tissue lysate, HUVEC, MCF7, K-562.

Subcellular location: Golgi apparatus membrane.

Database links: SwissProt: Q8NES3 Human | O09010 Mouse

Recommended Dilutions:

WB	1:1,000
IF-Cell	1:500
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

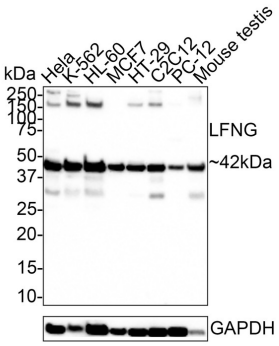

Fig1: Western blot analysis of LFNG on different lysates with Rabbit anti-LFNG antibody (HA721367) at 1/1,000 dilution.

Lane 1: Hela cell lysate (15 µg/Lane)
Lane 2: K-562 cell lysate (15 µg/Lane)
Lane 3: HL-60 cell lysate (15 µg/Lane)
Lane 4: MCF7 cell lysate (15 µg/Lane)
Lane 5: HT-29 cell lysate (15 µg/Lane)
Lane 6: C2C12 cell lysate (15 µg/Lane)
Lane 7: PC-12 cell lysate (15 µg/Lane)
Lane 8: Mouse testis tissue lysate (30 µg/Lane)

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

Exposure time: 7 seconds;

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 (HA721367) at 1/1,000 dilution was used in 5% NFDM/TBST at room temperature for 2 hours. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1:100,000 dilution was used for 1 hour at room temperature.

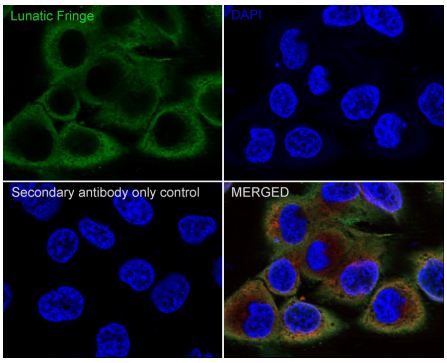

Fig2: Immunocytochemistry analysis of HUVEC cells labeling LFNG with Rabbit anti-LFNG antibody (HA721367) at 1/500 dilution.

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-LFNG antibody (HA721367) at 1/200 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. Nuclear DNA was labelled in blue with DAPI.

Beta tubulin (M1305-2, red) was stained at 1/200 dilution overnight at +4℃. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

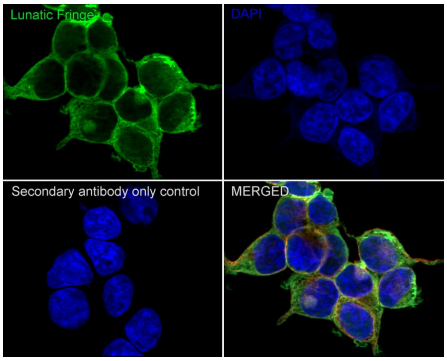

Fig3: Immunocytochemistry analysis of MCF7 cells labeling LFNG with Rabbit anti-LFNG antibody (HA721367) at 1/500 dilution.

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-LFNG antibody (HA721367) at 1/200 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. Nuclear DNA was labelled in blue with DAPI.

Beta tubulin (M1305-2, red) was stained at 1/200 dilution overnight at +4℃. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

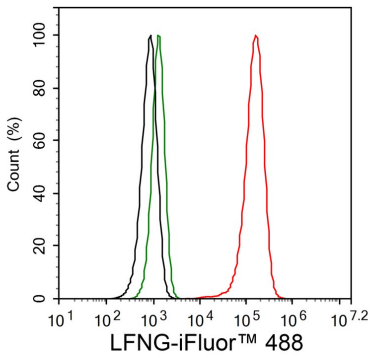

Fig4: Flow cytometric analysis of K-562 cells labeling LFNG.

Cells were fixed and permeabilized. Then stained with the primary antibody (HA721367, 1ug/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).

Background References

1. K Shimizu et al. Manic fringe and lunatic fringe modify different sites of the Notch2 extracellular region, resulting in different signaling modulation. J Biol Chem 276(28):25753-8 (2001)
2. Riley MF et al. Mir-125a-5p-mediated regulation of Lfng is essential for the avian segmentation clock. Dev Cell 24(5) (2013)