Anti-DDIT3 Antibody [PSH07-65]

HA722854

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IF-Cell, FC
Molecular Wt:	Predicted band size: 19 kDa
Clone number:	PSH07-65

Description: DNA damage-inducible transcript 3, also known as C/EBP homologous protein (CHOP), is a pro-apoptotic transcription factor that is encoded by the DDIT3 gene. It is a member of the CCAAT/enhancer-binding protein (C/EBP) family of DNA-binding transcription factors. The protein functions as a dominant-negative inhibitor by forming heterodimers with other C/EBP members, preventing their DNA binding activity. The protein is implicated in adipogenesis and erythropoiesis and has an important role in the cell's stress response.

Immunogen: Recombinant protein within human DDIT3 aa 1-169.

Positive control: HeLa treated with 2μg/mL tunicamycin for 8 hours cell lysate, C2C12 treated with 2μg/mL thapsigargin for 8 hours cell lysate, C6 cell lysate, C6 treated with 2μg/mL tunicamycin for 8 hours cell lysate, HeLa cells treated with 2μg/mL tunicamycin for 8 hours, C2C12 cells treated with 2μg/mL thapsigargin for 8 hours, C6 cells treated with 2μg/mL tunicamycin for 8 hours.

Subcellular location: Cytoplasm, Nucleus.

Database links: SwissProt: P35638 Human | P35639 Mouse | Q62857 Rat

Recommended Dilutions:

WB	1:5,000
IF-Cell	1:100
FC	1:1,000

Storage Buffer: PBS (pH7.4), 0.1% BSA, 40% Glycerol. Preservative: 0.05% Sodium Azide.

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

Purity: Protein A affinity purified.

Images

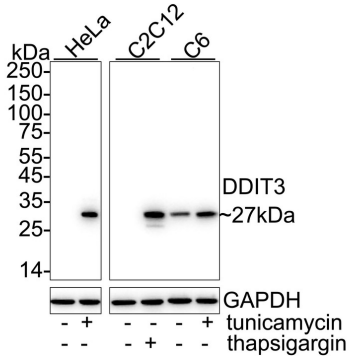

Fig1: Western blot analysis of DDIT3 on different lysates with Rabbit anti-DDIT3 antibody (HA722854) at 1/5,000 dilution.

Lane 1: HeLa cell lysate
Lane 2: HeLa treated with 2μg/mL tunicamycin for 8 hours cell lysate
Lane 3: C2C12 cell lysate
Lane 4: C2C12 treated with 2μg/mL thapsigargin for 8 hours cell lysate
Lane 5: C6 cell lysate
Lane 6: C6 treated with 2μg/mL tunicamycin for 8 hours cell lysate

Lysates/proteins at 20 μg/Lane.

Predicted band size: 19 kDa
Observed band size: 27 kDa

Exposure time: Lane 1-2: 3 minutes; Lane 3-6: 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 (HA722854) at 1/5,000 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.

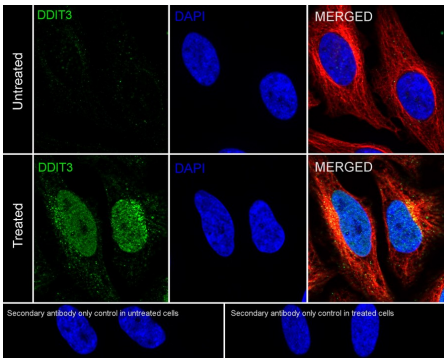

Fig2: Immunocytochemistry analysis of HeLa cells treated with 2μg/mL tunicamycin for 8 hours labeling DDIT3 with Rabbit anti-DDIT3 antibody (HA722854) at 1/100 dilution.

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-DDIT3 antibody (HA722854) 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.

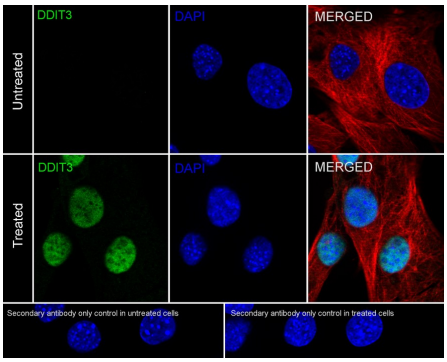

Fig3: Immunocytochemistry analysis of C2C12 cells treated with 2μg/mL thapsigargin for 8 hours labeling DDIT3 with Rabbit anti-DDIT3 antibody (HA722854) at 1/100 dilution.

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-DDIT3 antibody (HA722854) 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.

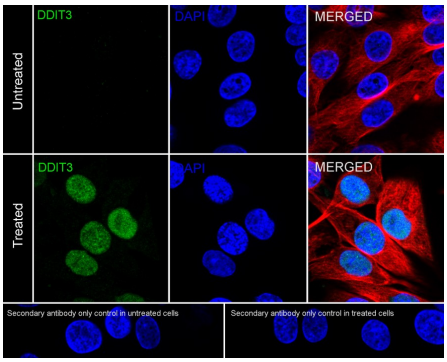

Fig4: Immunocytochemistry analysis of C6 cells treated with 2μg/mL tunicamycin for 8 hours labeling DDIT3 with Rabbit anti-DDIT3 antibody (HA722854) at 1/100 dilution.

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-DDIT3 antibody (HA722854) 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.

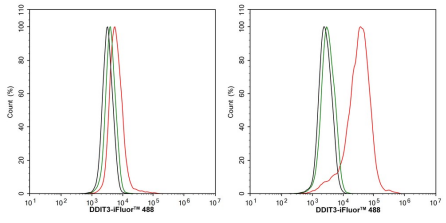

Fig5: Flow cytometric analysis of C2C12 cells treated with 2μg/mL thapsigargin for 8 hours labeling DDIT3.

Cells were fixed and permeabilized. Then stained with the primary antibody (HA722854, 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).

Background References

1. Li M et al. DDIT3 Directs a Dual Mechanism to Balance Glycolysis and Oxidative Phosphorylation during Glutamine Deprivation. Adv Sci (Weinh). 2021 Jun
2. Wang Y et al. DDIT3 aggravates pulpitis by modulating M1 polarization through EGR1 in macrophages. Int Immunopharmacol. 2023 Jul