Anti-DDIT3 Antibody [JM10-31]

ET1703-05

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IHC-P, FC
Molecular Wt:	Predicted band size: 19 kDa
Clone number:	JM10-31

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. The regulation of CHOP expression plays an important role in metabolic diseases and in some cancers through its function in mediating apoptosis. The regulation of CHOP expression could be a potential approach to affecting cancer cells through the induction of apoptosis. In the intestinal epithelium, CHOP has been demonstrated to be downregulated under inflammatory conditions (in inflammatory bowel diseases and experimental models of colitis). In this context, CHOP seems to rather regulate the cell cycle than apoptotic processes. Mutations or fusions of CHOP (e.g. with FUS to form FUS-CHOP) can cause Myxoid liposarcoma.

Immunogen: Synthetic peptide within Human DDIT3 aa 135-169 / 169.

Positive control: SW480 cell lysate, HeLa cell lysate, LOVO cell lysate, PC-12 cell lysate, human breast carcinoma tissue, mouse pancreas tissue, human stomach carcinoma tissue, human pancreas tissue, mouse brain tissue, Hela.

Subcellular location: Cytoplasm, Nucleus.

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

Recommended Dilutions:

WB	1:5,000
IHC-P	1:50-1:200
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

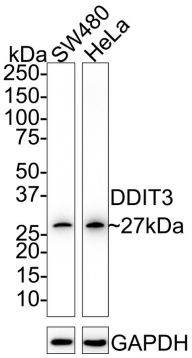

Fig1: Western blot analysis of DDIT3 on different lysates with Rabbit anti-DDIT3 antibody (ET1703-05) at 1/5,000 dilution.

Lane 1: SW480 cell lysate (20 µg/Lane)
Lane 2: HeLa cell lysate (20 µg/Lane)

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

Exposure time: 3 minutes;

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 (ET1703-05) 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.

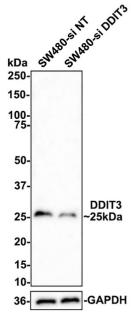

Fig2: Western blot analysis of DDIT3 on different lysates with Rabbit anti-DDIT3 antibody (ET1703-05) at 1/1,000 dilution.

Lane 1: SW480-si NT cell lysate
Lane 2: SW480-si DDIT3 cell lysate

Lysates/proteins at 3 µg/Lane.

Predicted band size: 19 kDa
Observed band size: 25 kDa

Exposure time: 5 minutes;

4-20% SDS-PAGE gel.

ET1703-05 was shown to specifically react with DDIT3 in SW480-si NT cells. Weakened band was observed when SW480-si DDIT3 sample was tested. SW480-si NT and SW480-si DDIT3 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 antibody (ET1703-05, 1/1,000) and Loading control antibody (Rabbit anti-GAPDH, ET1601-4, 1/10,000) were used in 5% BSA 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.

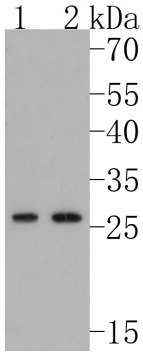

Fig3: Western blot analysis of DDIT3 on different lysates. Proteins were transferred to a PVDF membrane and blocked with 5% BSA in PBS for 1 hour at room temperature. The primary antibody (ET1703-05, 1/500) was used in 5% BSA at room temperature for 2 hours. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1:200,000 dilution was used for 1 hour at room temperature.
Positive control:
Lane 1: LOVO cell lysate
Lane 2: PC-12 cell lysate

Predicted band size: 19 kDa
Observed band size: 25 kDa

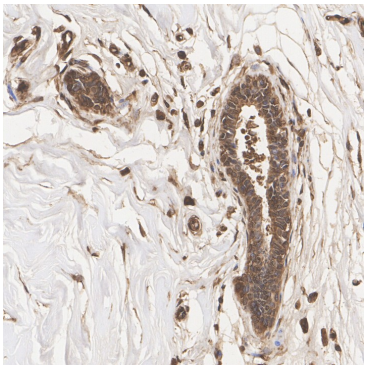

Fig4: Immunohistochemical analysis of paraffin-embedded human breast cancer tissue with Rabbit anti-DDIT3 antibody (ET1703-05) at 1/1,000 dilution.

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 (ET1703-05) 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.

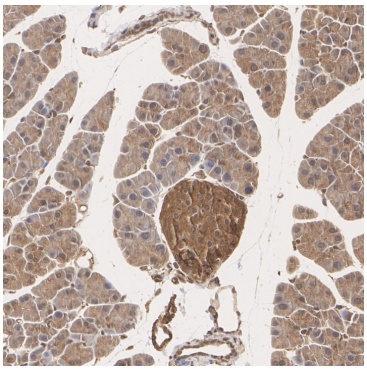

Fig5: Immunohistochemical analysis of paraffin-embedded mouse pancreas tissue with Rabbit anti-DDIT3 antibody (ET1703-05) at 1/1,000 dilution.

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 (ET1703-05) 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.

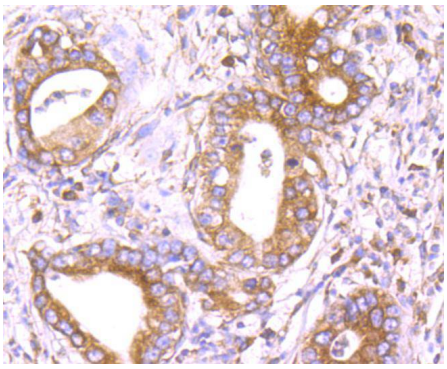

Fig6: Immunohistochemical analysis of paraffin-embedded human stomach carcinoma tissue using anti-DDIT3 antibody. The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes.The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1703-05, 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.

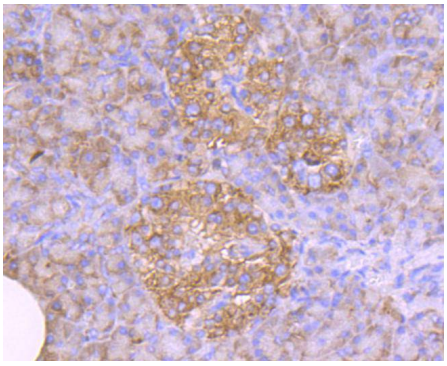

Fig7: Immunohistochemical analysis of paraffin-embedded human pancreas tissue using anti-DDIT3 antibody. The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes.The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1703-05, 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.

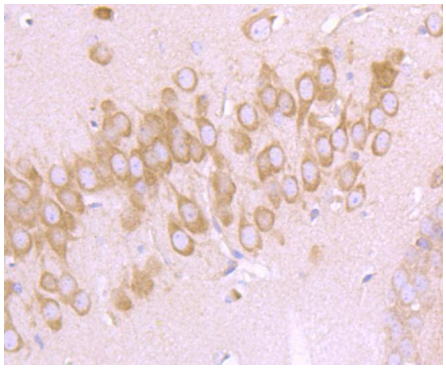

Fig8: Immunohistochemical analysis of paraffin-embedded mouse brain tissue using anti-DDIT3 antibody. The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes.The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1703-05, 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.

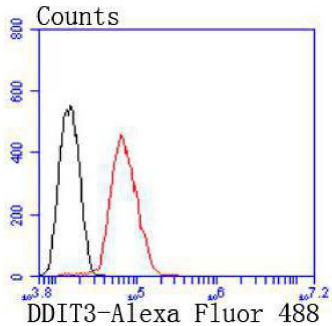

Fig9: Flow cytometric analysis of DDIT3 was done on Hela cells. The cells were fixed, permeabilized and stained with the primary antibody (ET1703-05, 1/50) (red). After incubation of the primary antibody at room temperature for an hour, the cells were stained with a Alexa Fluor®488 conjugate-Goat anti-Rabbit IgG Secondary antibody at 1/1,000 dilution for 30 minutes.Unlabelled sample was used as a control (cells without incubation with primary antibody; black).

Background References

1. Sun XY et al. Valproate attenuates diabetic nephropathy through inhibition of endoplasmic reticulum stress-induced apoptosis. Mol Med Rep 13:661-8 (2016).
2. Greenwood M et al. Transcription Factor CREB3L1 Regulates Endoplasmic Reticulum Stress Response Genes in the Osmotically Challenged Rat Hypothalamus. PLoS One 10:e0124956 (2015).