Anti-Phospho-HSF1 (S326) Antibody [SU31-03] ET1608-11

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human
Applications:	WB, IHC-P, IP
Molecular Wt:	Predicted band size: 57 kDa
Clone number:	SU31-03

Description: Heat shock factor 1 (HSF1) is a protein that in humans is encoded by the HSF1 gene. HSF1 is highly conserved in eukaryotes and is the primary mediator of transcriptional responses to proteotoxic stress with important roles in non-stress regulation such as development and metabolism. The HSF1 protein regulates the heat shock response (HSR) pathway in humans by acting as the major transcription factor for heat shock proteins. The HSR plays a protective role by ensuring proper folding and distribution of proteins within cells. This pathway is induced by not only temperature stress, but also by a variety of other stressors such as hypoxic conditions and exposure to contaminants. HSF1 transactivates genes for many cytoprotective proteins involved in heat shock, DNA damage repair, and metabolism. This illustrates the versatile role of HSF1 in not only the heat shock response, but also in aging and diseases.

Immunogen: Synthetic phospho-peptide corresponding to residues surrounding Ser326 of Human HSF1 aa 301-350 / 529.

Positive control: HeLa 42℃ heat shocked for 30 minutes cell lysate, Hela, AGS, MCF-7, human breast tissue, human tonsil tissue, human lung tissue, human thyroid tissue, human skin tissue, human pancreas tissue, rat bladder tissue.

Subcellular location: Cytoplasm, Nucleus.

Database links: SwissProt: Q00613 Human

Recommended Dilutions:

WB	1:1,000-1:2,000
IHC-P	1:50-1:200
IP	1-2μg/sample

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

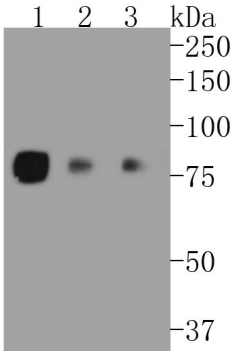

Fig1: Western blot analysis of Phospho-HSF1 (S326) on different lysates. Proteins were transferred to a PVDF membrane and blocked with 5% BSA in PBS for 1 hour at room temperature. The primary antibody (ET1608-11, 1/500) was used in 5% BSA at room temperature for 2 hours. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1:5,000 dilution was used for 1 hour at room temperature.
Positive control:
Lane 1: Hela cell lysate
Lane 2: BT-20 cell lysate
Lane 3: MCF-7 cell lysate

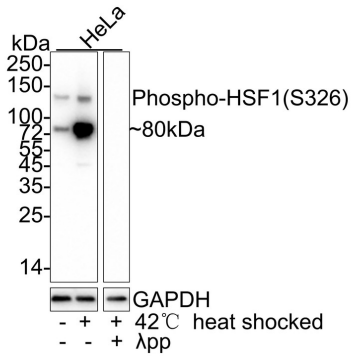

Fig2: Western blot analysis of Phospho-HSF1 (S326) on different lysates with Rabbit anti-Phospho-HSF1 (S326) antibody (ET1608-11) at 1/1,000 dilution.

Lane 1: HeLa cell lysate
Lane 2: HeLa 42℃ heat shocked for 30 minutes cell lysate
Lane 3: HeLa 42℃ heat shocked for 30 minutes cell lysate, then the membrane treated with λpp for 1 hour

Lysates/proteins at 20 µg/Lane.

Predicted band size: 57 kDa
Observed band size: 80 kDa

Exposure time: 1 minute 59 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 (ET1608-11) 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.

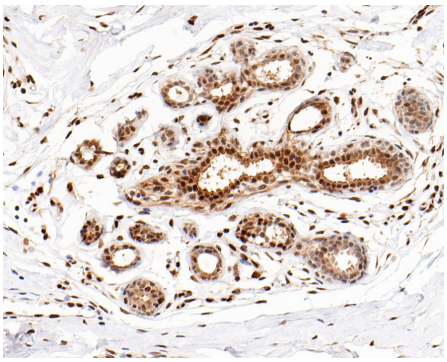

Fig3: Immunohistochemical analysis of paraffin-embedded human breast tissue with Rabbit anti-Phospho-HSF1 (S326) antibody (ET1608-11) at 1/800 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) (high pressure) for 2 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 (ET1608-11) at 1/800 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

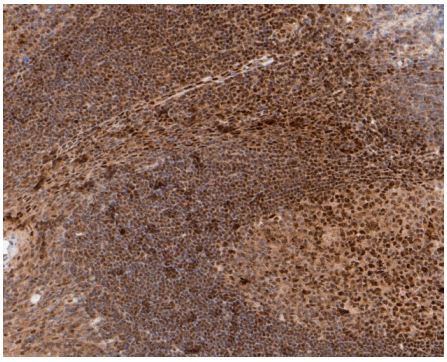

Fig4: Immunohistochemical analysis of paraffin-embedded human tonsil tissue using anti-Phospho-HSF1 (S326) antibody. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) (high pressure) for 2 minutes. The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1608-11, 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.

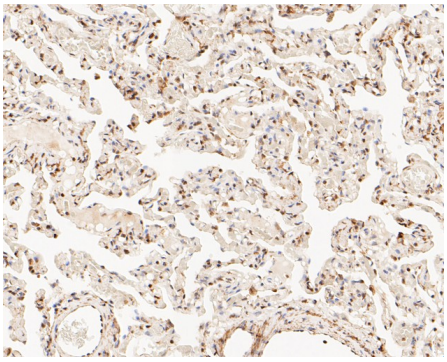

Fig5: Immunohistochemical analysis of paraffin-embedded human lung tissue using anti-Phospho-HSF1 (S326) antibody. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) (high pressure) for 2 minutes. The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1608-11, 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.

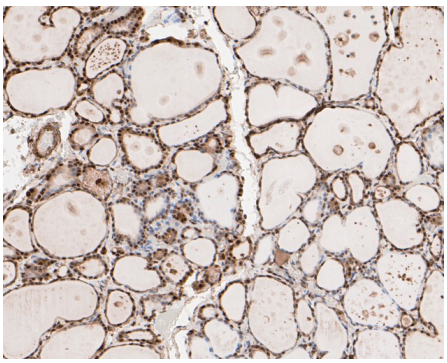

Fig6: Immunohistochemical analysis of paraffin-embedded human thyroid tissue using anti-Phospho-HSF1 (S326) antibody. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) (high pressure) for 2 minutes. The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1608-11, 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.

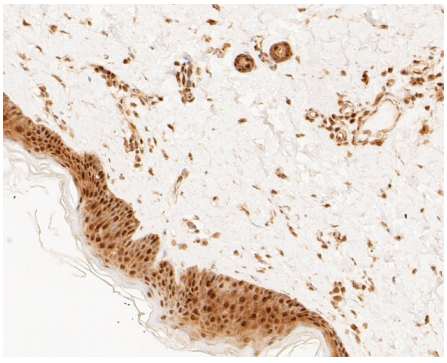

Fig7: Immunohistochemical analysis of paraffin-embedded human skin tissue using anti-Phospho-HSF1 (S326) antibody. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) (high pressure) for 2 minutes. The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1608-11, 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.

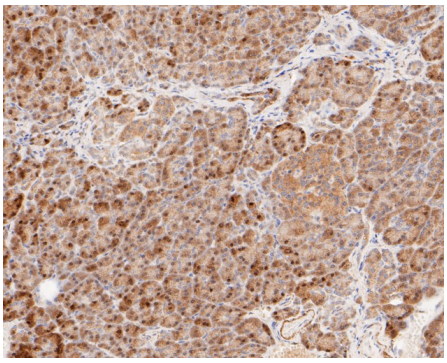

Fig8: Immunohistochemical analysis of paraffin-embedded human pancreas tissue using anti-Phospho-HSF1 (S326) antibody. The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) (high pressure) for 2 minutes. The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH_2O and PBS, and then probed with the primary antibody (ET1608-11, 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.

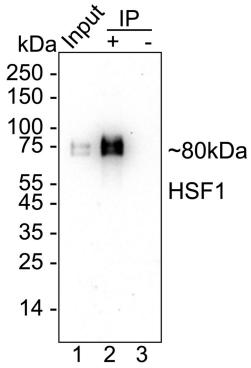

Fig9: Phospho-HSF1 (S326) was immunoprecipitated from 0.2 mg HeLa cell lysate with ET1608-11 at 2 µg/10 µl beads. Western blot was performed from the immunoprecipitate using ET1608-11 at 1/1,000 dilution. Anti-Rabbit IgG for IP Nano-secondary antibody (NBI01H) at 1/5,000 dilution was used for 1 hour at room temperature.

Lane 1: HeLa cell lysate (input)
Lane 2: ET1608-11 IP in HeLa cell lysate
Lane 3: Rabbit IgG instead of ET1608-11 in HeLa cell lysate

Blocking/Dilution buffer: 5% NFDM/TBST
Exposure time: 3 minutes; ECL: K1801

Background References

1. Carpenter RL et al. Akt phosphorylates and activates HSF-1 independent of heat shock, leading to Slug overexpression and epithelial-mesenchymal transition (EMT) of HER2-overexpressing breast cancer cells. Oncogene N/A:N/A (2014).
2. Schulz R et al. HER2/ErbB2 activates HSF1 and thereby controls HSP90 clients including MIF in HER2-overexpressing breast cancer. Cell Death Dis 5:e980 (2014).