Anti-SIRT1 Antibody

ER130811

Product Type:	Rabbit polyclonal IgG, primary antibodies
Species reactivity:	Human, Mouse
Applications:	WB, IHC-P, FC, IF-Cell
Molecular Wt:	Predicted band size: 82 kDa

Description: SirT1, the mammalian ortholog of Sir2, is a nuclear protein implicated in the regulation of many cellular processes, including apoptosis, cellular senescence, endocrine signaling, glucose homeostasis, aging, and longevity. Targets of SirT1 include acetylated p53, p300, Ku70, forkhead (FoxO) transcription factors, PPARγ, and the PPARγ coactivator-1α (PGC-1α) protein. Deacetylation of p53 and FoxO transcription factors represses apoptosis and increases cell survival. SirT1 deacetylase activity is inhibited by nicotinamide and activated by resveratrol.

Immunogen: Synthetic peptide within Human SIRT1 aa 698-747 / 747.

Positive control: Hela cell lysate, Jurkat cell lysate, F9 cell lysate, HeLa, F9, human colon carcinoma tissue, human lung carcinoma tissue, mouse liver tissue, mouse testis tissue.

Subcellular location: Nucleus, cytoplasm, Mitochondrion.

Database links: SwissProt: Q96EB6 Human

Recommended Dilutions:

WB	1:1,000-1:2,000
IHC-P	1:200
FC	1:1,000
IF-Cell	1:100

Storage Buffer: 1*PBS (pH7.4), 0.2% 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: Immunogen affinity purified.

Images

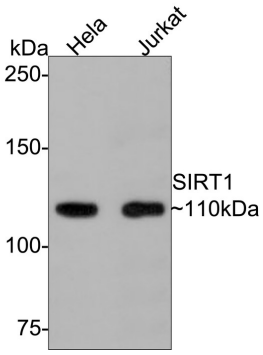

Fig1: Western blot analysis of SIRT1 on different lysates with Rabbit anti-SIRT1 antibody (ER130811) at 1/500 dilution.

Lane 1: Hela cell lysate
Lane 2: Jurkat cell lysate

Lysates/proteins at 10 µg/Lane.

Predicted band size: 82 kDa
Observed band size: 110 kDa

Exposure time: 2 minutes;

6% 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 (ER130811) at 1/500 dilution was used in 5% NFDM/TBST at room temperature for 2 hours. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1:300,000 dilution was used for 1 hour at room temperature.

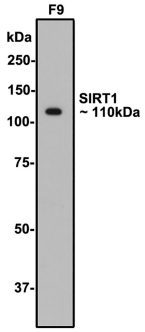

Fig2: Western blot analysis of SIRT1 on F9 cell lysates with Rabbit anti-SIRT1 antibody (ER130811) at 1/2,000 dilution.

Lysates/proteins at 10 µg/Lane.

Predicted band size: 82 kDa
Observed band size: 110 kDa

Exposure time: 1 minute;

8% 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 (ER130811) at 1/2,000 dilution was used in 5% NFDM/TBST at room temperature for 2 hours. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1:300,000 dilution was used for 1 hour at room temperature.

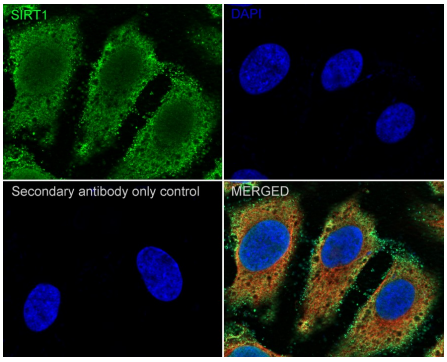

Fig3: Immunocytochemistry analysis of HeLa cells labeling SIRT1 with Rabbit anti-SIRT1 antibody (ER130811) at 1/100 dilution.

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-SIRT1 antibody (ER130811) 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.

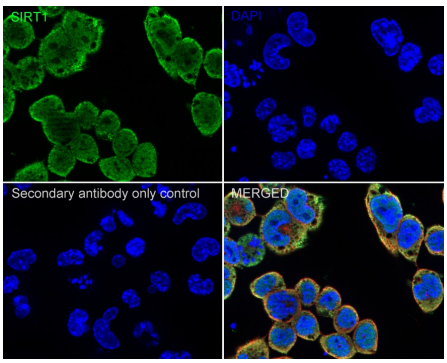

Fig4: Immunocytochemistry analysis of F9 cells labeling SIRT1 with Rabbit anti-SIRT1 antibody (ER130811) at 1/100 dilution.

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-SIRT1 antibody (ER130811) 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.

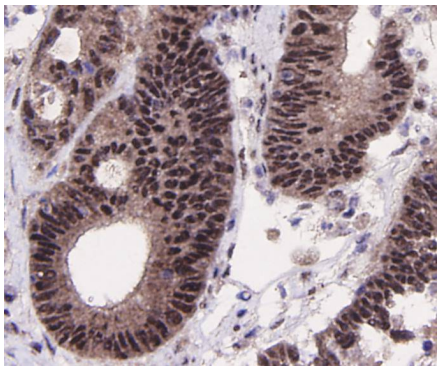

Fig5: Immunohistochemical analysis of paraffin-embedded human colon carcinoma tissue using anti-SIRT1 antibody. Counter stained with hematoxylin.

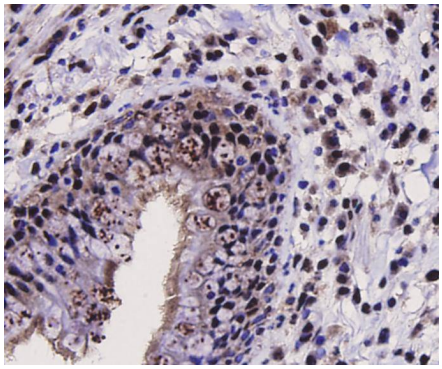

Fig6: Immunohistochemical analysis of paraffin-embedded human lung carcinoma tissue using anti-SIRT1 antibody. Counter stained with hematoxylin.

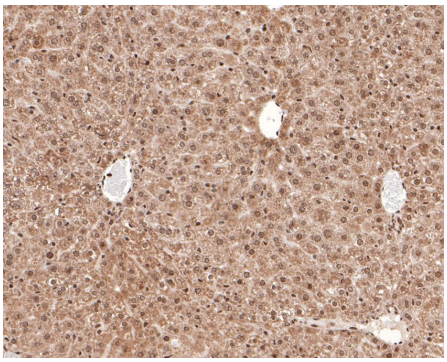

Fig7: Immunohistochemical analysis of paraffin-embedded mouse liver tissue with Rabbit anti-SIRT1 antibody (ER130811) at 1/200 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) 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 (ER130811) at 1/200 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.

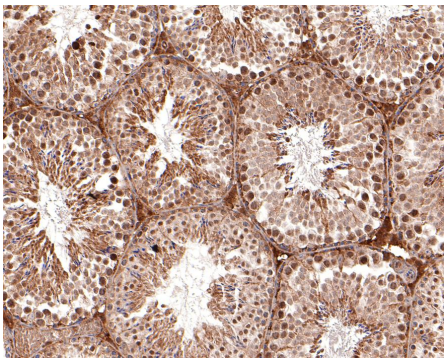

Fig8: Immunohistochemical analysis of paraffin-embedded mouse testis tissue with Rabbit anti-SIRT1 antibody (ER130811) at 1/200 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) 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 (ER130811) at 1/200 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.

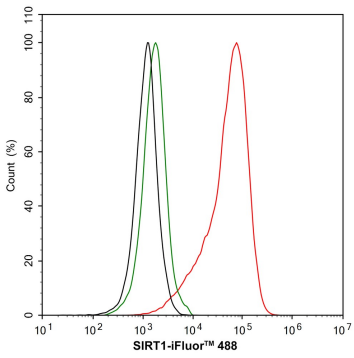

Fig9: Flow cytometric analysis of F9 cells labeling SIRT1.

Cells were fixed and permeabilized. Then stained with the primary antibody (ER130811, 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).

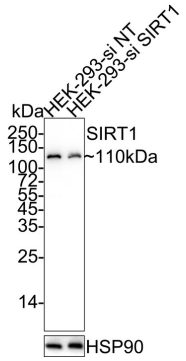

Fig10: Western blot analysis of SIRT1 on different lysates with Rabbit anti-SIRT1 antibody (ER130811) at 1/20,000 dilution.

Lane 1: HEK-293-si NT cell lysate
Lane 2: HEK-293-si SIRT1 cell lysate

Lysates/proteins at 10 µg/Lane.

Predicted band size: 82 kDa
Observed band size: 110 kDa

Exposure time: 50 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 (ER130811) at 1/20,000 dilution was used in 5% NFDM/TBST at 4℃ overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/100,000 dilution was used for 1 hour at room temperature.

Background References

1. "Human Sir2-related protein SIRT1 associates with the bHLH repressors HES1 and HEY2 and is involved in HES1- and HEY2-mediated transcriptional repression." Takata T., Ishikawa F. Biochem. Biophys. Res. Commun. 301:250-257(2003)
2. "Inhibition of silencing and accelerated aging by nicotinamide, a putative negative regulator of yeast sir2 and human SIRT1." Bitterman K.J., Anderson R.M., Cohen H.Y., Latorre-Esteves M., Sinclair D.A. J. Biol. Chem. 277:45099-45107(2002)
3. "Human SirT1 interacts with histone H1 and promotes formation of facultative heterochromatin." Vaquero A., Scher M., Lee D., Erdjument-Bromage H., Tempst P., Reinberg D.Mol. Cell 16:93-105(2004)
4. "Human immunodeficiency virus type 1 Tat protein inhibits the SIRT1 deacetylase and induces T cell hyperactivation." Kwon H.S., Brent M.M., Getachew R., Jayakumar P., Chen L.F., Schnolzer M., McBurney M.W., Marmorstein R., Greene W.C., Ott M. Cell Host Microbe 3:158-167(2008)