Anti-SIRT1 Antibody [SZ04-01]

ET1603-3

Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IF-Cell, IF-Tissue, IHC-P, IP, ChIP
Molecular Wt:	Predicted band size: 82 kDa
Clone number:	SZ04-01

Description: Sirtuin 1 is a member of the sirtuin family of proteins, homologs of the Sir2 gene in S. cerevisiae. Members of the sirtuin family are characterized by a sirtuin core domain and grouped into four classes. The functions of human sirtuins have not yet been determined; however, yeast sirtuin proteins are known to regulate epigenetic gene silencing and suppress recombination of rDNA. Studies suggest that the human sirtuins may function as intracellular regulatory proteins with mono-ADP-ribosyltransferase activity. The protein encoded by this gene is included in class I of the sirtuin family. Sirtuin 1 is downregulated in cells that have high insulin resistance and inducing its expression increases insulin sensitivity, suggesting the molecule is associated with improving insulin sensitivity. Furthermore, SIRT1 was shown to de-acetylate and affect the activity of both members of the PGC1-alpha/ERR-alpha complex, which are essential metabolic regulatory transcription factors.

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

Positive control: HeLa cell lysate, HEK-293 cell lysate, A549 cell lysate, F9 cell lysate, Mouse testis tissue lysate, Rat testis tissue lysate, HEK-293, human testis tissue, mouse testis tissue, rat testis tissue.

Subcellular location: Nucleus, Cytoplasm, Mitochondrion.

Database links: SwissProt: Q96EB6 Human | Q923E4 Mouse
Entrez Gene: 309757 Rat

Recommended Dilutions:

WB	1:2,000
IF-Cell	1:50-1:200
IF-Tissue	1:50-1:200
IHC-P	1:50-1:1,000
IP	Use at an assay dependent concentration.
ChIP	Use 0.5~2 μg for 25 μg of chromatin.

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

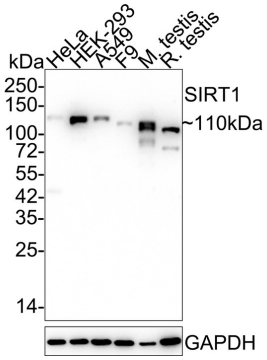

Fig1: Western blot analysis of SIRT1 on different lysates with Rabbit anti-SIRT1 antibody (ET1603-3) at 1/2,000 dilution.

Lane 1: HeLa cell lysate (15 µg/Lane)
Lane 2: HEK-293 cell lysate (15 µg/Lane)
Lane 3: A549 cell lysate (15 µg/Lane)
Lane 4: F9 cell lysate (15 µg/Lane)
Lane 5: Mouse testis tissue lysate (20 µg/Lane)
Lane 6: Rat testis tissue lysate (20 µg/Lane)

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

Exposure time: 2 minutes 37 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 (ET1603-3) at 1/2,000 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.

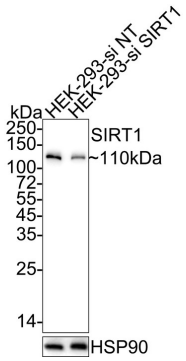

Fig2: Western blot analysis of SIRT1 on different lysates with Rabbit anti-SIRT1 antibody (ET1603-3) at 1/20,000 dilution.

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 (ET1603-3) 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.

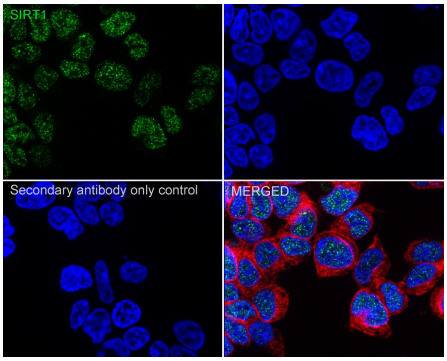

Fig3: Immunocytochemistry analysis of HEK-293 cells labeling SIRT1 with Rabbit anti-SIRT1 antibody (ET1603-3) at 1/100 dilution.

Cells were fixed in 4% paraformaldehyde for 15 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 15 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 (ET1603-3) 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 (HA601187, 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.

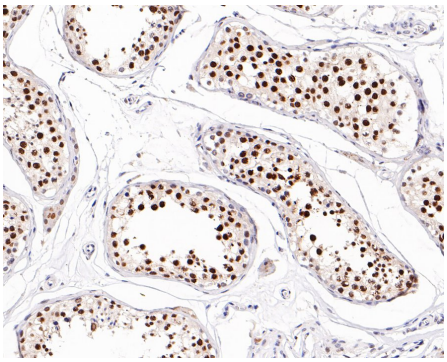

Fig4: Immunohistochemical analysis of paraffin-embedded human testis tissue with Rabbit anti-SIRT1 antibody (ET1603-3) at 1/1,000 dilution.

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 (ET1603-3) 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.

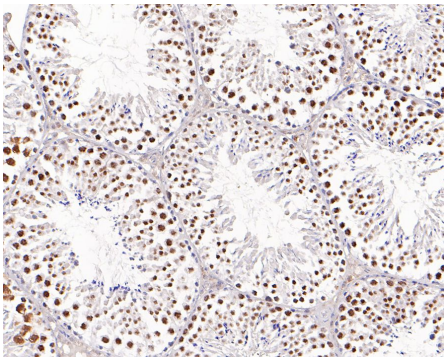

Fig5: Immunohistochemical analysis of paraffin-embedded mouse testis tissue with Rabbit anti-SIRT1 antibody (ET1603-3) at 1/1,000 dilution.

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 (ET1603-3) 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.

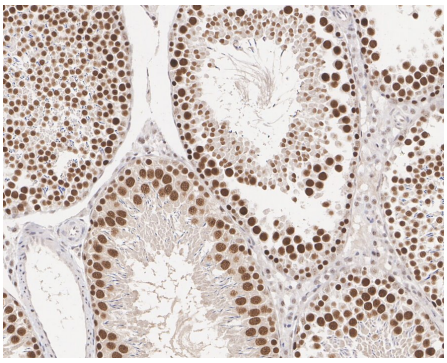

Fig6: Immunohistochemical analysis of paraffin-embedded rat testis tissue with Rabbit anti-SIRT1 antibody (ET1603-3) at 1/1,000 dilution.

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 (ET1603-3) 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.

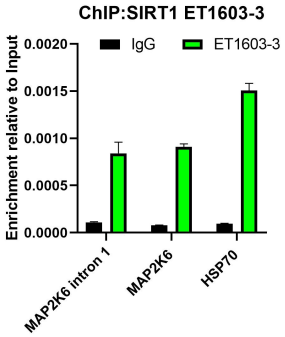

Fig7: Chromatin immunoprecipitations were performed with cross-linked chromatin from HeLa cells with SIRT1 (ET1603-3) or Normal Rabbit IgG according to the ChIP protocol. The enriched DNA was quantified by real-time PCR using indicated primers. The amount of immunoprecipitated DNA in each sample is represented as signal relative to the total amount of input chromatin, which is equivalent to one.

Note: All products are "FOR RESEARCH USE ONLY AND ARE NOT INTENDED FOR DIAGNOSTIC OR THERAPEUTIC USE".

Background References

1. Kang K et al. Carnosic acid slows photoreceptor degeneration in the Pde6b(rd10) mouse model of retinitis pigmentosa. Sci Rep 6:22632 (2016).
2. Zheng YC et al. 1,2,3-Triazole-Dithiocarbamate Hybrids, a Group of Novel Cell Active SIRT1 Inhibitors. Cell Physiol Biochem 38:185-93 (2016).