## Anti-Phospho-SIRT1 (T530) Antibody [JJ206-6] ET1701-27



Product Type: Recombinant Rabbit monoclonal IgG, primary antibodies

Species reactivity: Human

Applications: WB, IF-Cell, IF-Tissue, IHC-P, FC

Molecular Wt: Predicted band size: 82 kDa

Clone number: JJ206-6

**Description:** The silent information regulator (SIR2) family of genes are highly-conserved from prokaryotes to eukaryotes

and are involved in diverse processes, including transcriptional regulation, cell cycle progression, DNA-damage repair and aging. In S. cerevisiae, Sir2p deacetylates histones in an NAD-dependent manner, which regulates silencing at the telomeric, rDNA and silent mating-type loci. Sir2p is the founding member of a large family, designated sirtuins, which contain a conserved catalytic domain. The human homologs, which include SIRT1-7, are divided into four main branches: SIRT1-3 are class I, SIRT4 is class II, SIRT5 is class III and SIRT6-7 are class IV. SIRT1 has the closest homology to the yeast Sir2p and is widely expressed in fetal and adult tissues. SIRT1 is highly expressed in heart, brain and skeletal muscle, with low expression in lung and placenta. SIRT1 regulates the p53-dependent DNA damage response pathway by binding to and deacetylating p53, specifically

at Lys 382.

**Immunogen:** Synthetic phospho-peptide corresponding to residues surrounding Thr530 of Human SIRT1 aa 501-550 / 747.

Positive control: HepG2, Hela, 293, human kidney tissue.

Subcellular location: Nucleus, Cytoplasm.

**Database links:** SwissProt Q96EB6 Human

**Recommended Dilutions:** 

 WB
 1:500

 IF-Cell
 1:100-1:500

 IF-Tissue
 1:100-1:500

 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% SodiumAzide.

**Storage Instruction:** Store at +4  $^{\circ}$ C after thawing. Aliquot store at -20  $^{\circ}$ C or -80  $^{\circ}$ C. Avoid repeated freeze / thaw cycles.

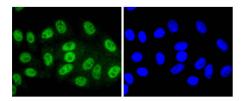
**Purity:** Protein A affinity purified.

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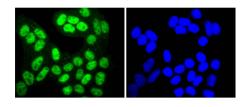


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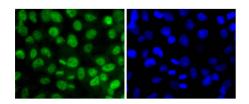




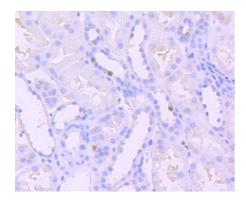
**Fig1:** ICC staining of Phospho-SIRT1 (T530) in HepG2 cells (green). Formalin fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 10 minutes at room temperature and blocked with 1% Blocker BSA for 15 minutes at room temperature. Cells were probed with the primary antibody (ET1701-27, 1/50) for 1 hour at room temperature, washed with PBS. Alexa Fluor®488 Goat anti-Rabbit IgG was used as the secondary antibody at 1/1,000 dilution. The nuclear counter stain is DAPI (blue).



**Fig2:** ICC staining of Phospho-SIRT1 (T530) in Hela cells (green). Formalin fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 10 minutes at room temperature and blocked with 1% Blocker BSA for 15 minutes at room temperature. Cells were probed with the primary antibody (ET1701-27, 1/50) for 1 hour at room temperature, washed with PBS. Alexa Fluor®488 Goat anti-Rabbit IgG was used as the secondary antibody at 1/1,000 dilution. The nuclear counter stain is DAPI (blue).



**Fig3:** ICC staining of Phospho-SIRT1 (T530) in 293 cells (green). Formalin fixed cells were permeabilized with 0.1% Triton X-100 in TBS for 10 minutes at room temperature and blocked with 1% Blocker BSA for 15 minutes at room temperature. Cells were probed with the primary antibody (ET1701-27, 1/50) for 1 hour at room temperature, washed with PBS. Alexa Fluor®488 Goat anti-Rabbit IgG was used as the secondary antibody at 1/1,000 dilution. The nuclear counter stain is DAPI (blue).



**Fig4:** Immunohistochemical analysis of paraffin-embedded human kidney tissue using anti-Phospho-SIRT1 (T530) antibody. The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 8.0-8.4) for 20 minutes. The tissues were blocked in 5% BSA for 30 minutes at room temperature, washed with ddH<sub>2</sub>O and PBS, and then probed with the primary antibody (ET1701-27, 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.

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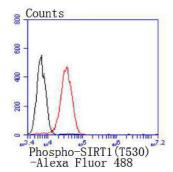


Fig5: Flow cytometric analysis of Phospho-SIRT1 (T530) was done on 293 cells. The cells were fixed, permeabilized and stained with the primary antibody (ET1701-27, 1/50) (red). After incubation of the primary antibody at room temperature for an hour, the cells were stained with a Alexa Fluor 488-conjugated 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).

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 refinitis 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).