Anti-SIRT1 pAb

Cat# CY-P1016

100 µg (0.5 mg/mL x 200 µL)

Clone Name	Applications	Species Cross-Reactivity	Molecular Wt.	Source Isotype
	IP, IF	H	100 kDa	Rabbit IgG

Background

Sir2 is a conserved protein and was recently shown to regulate lifespan extension both in budding yeast and nematode. In 2000, it was reported that the yeast Sir2 protein is a NAD(+)-dependent histone deacetylase that plays a critical role in transcriptional silencing, genome stability and longevity. A human homologue of Sir2, SIRT1, also functions as a NAD(+)-dependent-p53 deacetylase as well as a NAD(+)-dependent histone deacetylase. SIRT1 was shown to regulate the activity of the p53 tumor suppressor and inhibits apoptosis. These results have significant implications regarding an important role for SIRT1 in modulating the sensitivity of cells in p53-dependent apoptotic response and the possible effect in cancer therapy. Since the function of p53 is made to strengthen powerfully by using together with DNA damaging reagent, it is expected that inhibitor of SIRT1 becomes an effective anticancer drug.

Specificity/Sensitivity: SIRT1 Antibody detects endogenous levels of SIRT1 protein. The antibody does not cross-react with type I and II HDAC proteins.

Source/Purification: Polyclonal antibody is produced by immunizing rabbit with a recombinant full length SIRT. IgG is purified by affinity chromatography.

Recommended Antibody Dilutions: Immunoprecipitation: 1-2 µg /sample.

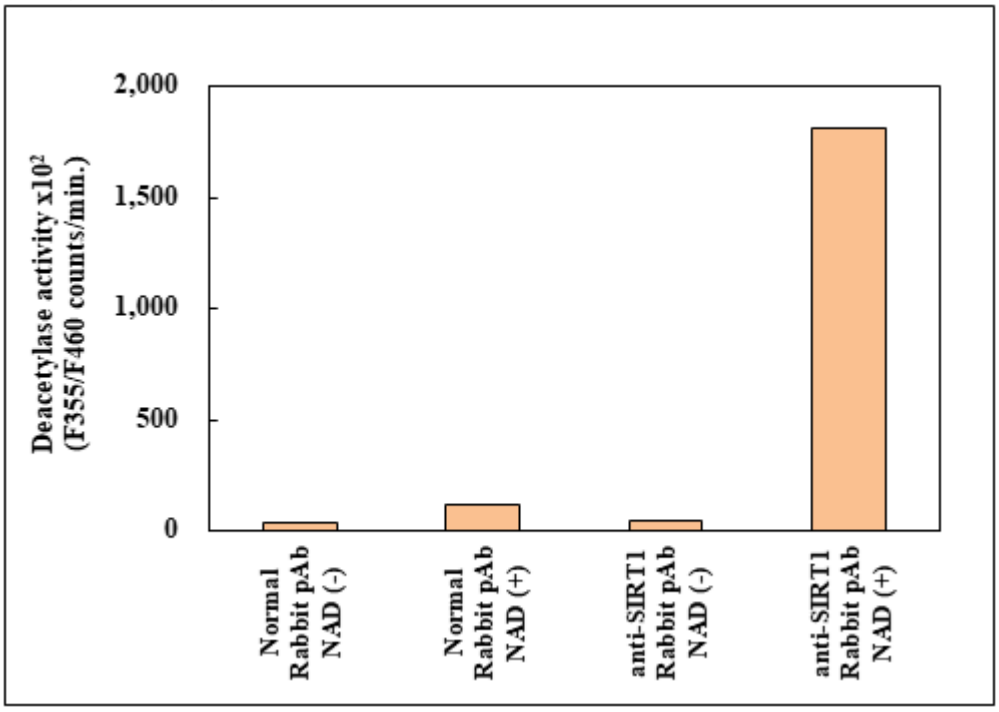
Storage: Supplied in 20 mM phosphate buffer (pH 7.5), 300 mM NaCl, 50 % glycerol. Store at -20°C.

Applications Key:
WB: Western blotting, **IP:** Immunoprecipitation, **IHC:** Immunohistochemistry, **IC:** Immunocytochemistry, **F:** Flow cytometry, **E:** ELISA, **FP:** Fluorescence polarization assay

Species Cross-Reactivity Key:
H: Human, **M:** Mouse, **R:** Rat, **Hm:** Hamster, **Mk:** Monkey, **Mi:** Mink, **C:** Chicken, **X:** Xenopus, **Z:** Zebra fish (Species enclosed in parentheses are predicted to react based on 100% sequence homology.)

General References:
1. Imai S, et al. Nature. 403, 795-800, 2000
2. Landry J et al. Proc Natl Acad Sci U S A 97, 5807-5811, 2000
3. Smith JS, et al. Proc Natl Acad Sci U S A 97, 6658-6663, 2000
4. Vaziri H, et al. Cell. 107, 149-159, 2001
5. Luo J et al. Cell. 107, 137-148, 2001
6. Langley E et al. EMBO J. 21, 2383-2396, 2002
7. Smith J. Trends Cell Biol. 12, 404, 2002
8. Grozinger CM, and Schreiber SL. Chem Biol, 9, 3-16, 2002

Fig.1 Measurement of 293T cell endogenous SIRT1 activity in an immunoprecipitate using anti-SIRT1 antibody by means of CycLex® SIRT1/Sir2 Deacetylase Fluorometric Assay Kit Ver.2 (CY-1151V2)



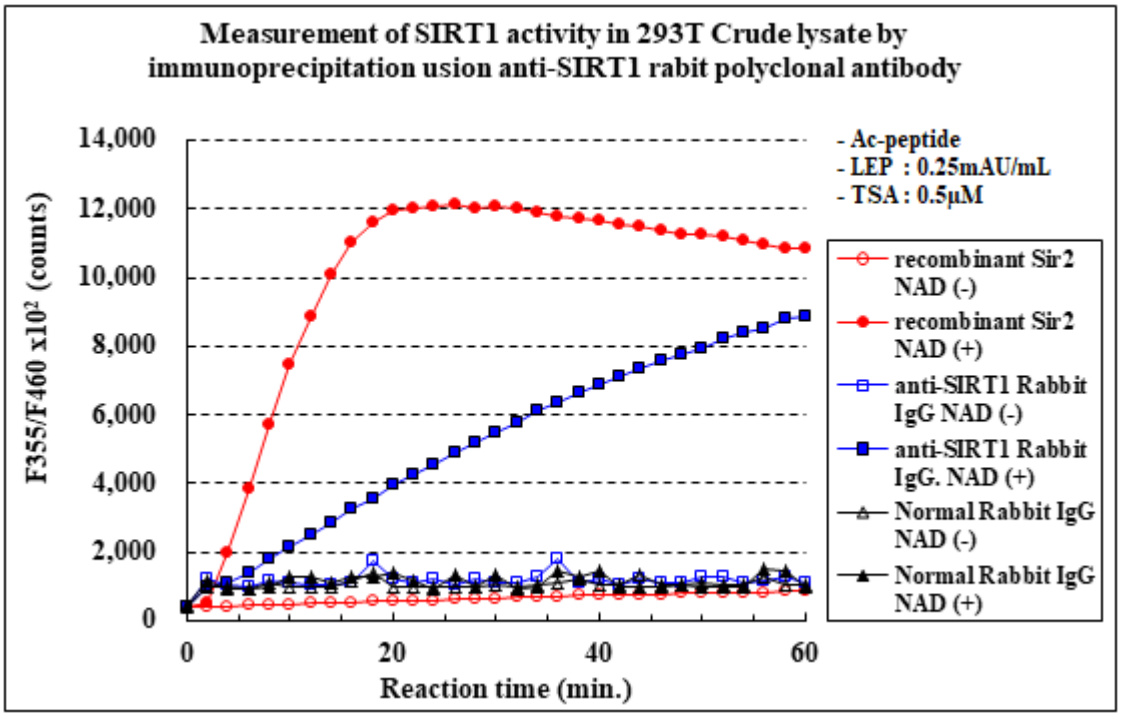
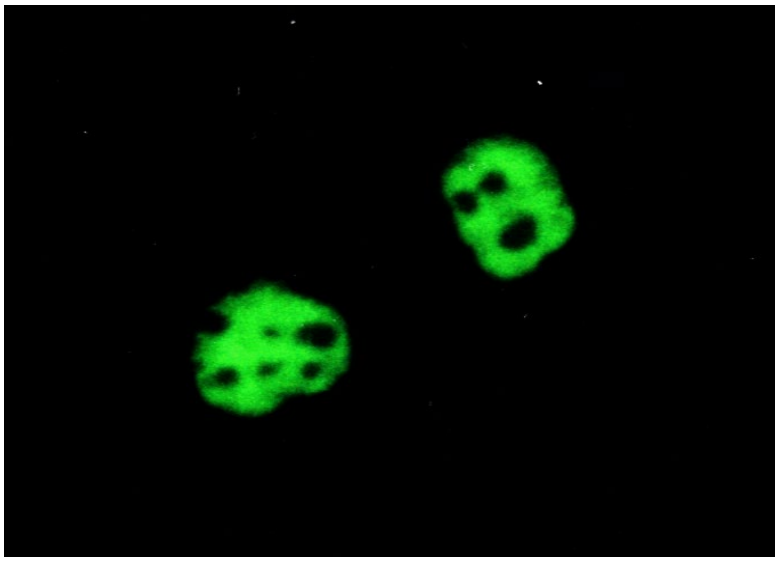


Fig.2 Subcellular localization of SIRT1 on 293T cell



Immunoprecipitation Followed by Measuring SITR1 Activity Protocol

Solutions and Reagents

Note: Prepare solutions with Milli-Q or equivalently purified water.

Cell Lysis Buffer (1X): 20 mM Tris (pH 7.5), 250 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1% Triton X-100, 1 mM DTT.

Protein A Agarose Beads: Add 5 mL of 1X PBS to 1.5 g of Protein A Agarose Beads. Shake 2 hours at 4°C; spin down. Wash the pellet twice with PBS. Resuspend beads in 1 volume of PBS. (Can be stored for 2 weeks at 4°C)

10X TBS (Tris-buffered saline): For 1 liter of 10X TBS, use 24.2 g Tris base and 80 g NaCl. Adjust pH to 7.6 with HCl (use at 1X).

Wash Buffer TBS/T: 1X TBS, 0.1% Tween-20

Preparing Cell Lysates

1. Aspirate media. Treat cells by adding fresh media containing regulator for desired time.
2. To harvest cells under nondenaturing conditions, remove media and rinse cells once with ice-cold PBS.
3. Remove PBS and add 0.5 mL 1X ice-cold Cell Lysis Buffer to each plate (10 cm^2) and incubate the plate on ice for 5 minutes.
4. Scrape cells off the plate and transfer to microcentrifuge tubes. Keep on ice.
5. Sonicate 4 times for 5 seconds each on ice.
6. Microcentrifuge for 10 minutes at 4°C, and transfer the supernatant to a new tube. The supernatant is the cell lysate. If necessary, lysate can be stored at –80°C.

Immunoprecipitation

1. Take 200 µL cell lysate and add primary antibody (1-2 µg; incubate with gentle rocking for 2 hrs or overnight at 4°C.
2. Add protein A agarose beads (20 µL of 50% bead slurry). Incubate with gentle rocking for 1–3 hours at 4°C.
3. Microcentrifuge for 30 seconds at 4°C. Wash pellet 3 times with 500 µL of 1X Cell Lysis Buffer and with Sir2 assay buffer (50 mM Tris-HCl, pH 8.8, 4 mM $MgCl_2$, 0.5 mM DTT). Keep on ice during washes.

4. After immunoprecipitation, add reaction mixture containing Fluoro-Substrate peptide solution to protein A agarose beads and measure NAD dependent deacetylase activity according to the procedure in CycLex® SIRT1/Sir2 Deacetylase Fluorometric Assay Kit Ver.2 (Cat# CY-1151V2).

For more information, please visit our web site**.**
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