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SIRT1 Polyclonal Antibody

Catalog Number PA5-17232

Product data sheet

Details	
Size	100 µL
Host/Isotope	Rabbit / IgG
Class	Polyclonal
Type	Antibody
Immunogen	Synthetic peptide corresponding to the carboxy terminus of human SirT1
Conjugate	Unconjugated
Form	Liquid
Concentration	21 µg/mL
Purification	Antigen affinity chromatography
Storage buffer	0.01M HEPES, pH 7.5, with 0.15M NaCl, 100µg/mL BSA, 50% glycerol
Contains	no preservative
Storage Conditions	-20°C

Species Reactivity	
Species reactivity	Human, Non-human primate
Published species	Not Applicable

Tested Applications	Dilution *
Immunoprecipitation (IP)	1:50
Western Blot (WB)	1:1,000
Immunocytochemistry (ICC/IF)	1:100

Published Applications	
Western Blot (WB)	See 1 publications below

* Suggested working dilutions are given as a guide only. It is recommended that the user titrate the product for use in their own experiment using appropriate negative and positive controls.

Product specific information

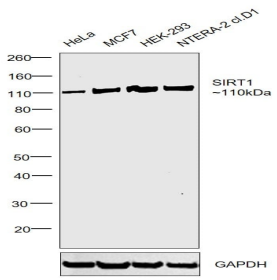
It is not recommended to aliquot this antibody. This antibody is not cross-reactive with other sirtuin proteins.

Background/Target Information

NAD-dependent protein deacetylase sirtuin-1 (SIRT1) links transcriptional regulation directly to intracellular energetics and participates in the coordination of several separated cellular functions such as cell cycle, repsonse to DNA damage, metabolism, apoptosis, and autophagy. SIRT1 can modulate chromatin function through deacetylation of histones and can promote alterations in the methylation of histone and DNA, leading to transcriptional repression. It is essential in skeletal muscle cell differentiation and in response to low nutrients mediates the inhibitory effect on skeletal myoblast differentiation which also involves 5'-AMP-activated protein kinase (AMPK) and nicotinamide phosphoribosyltransferase (NAMPT).

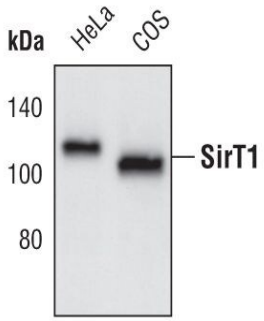
For Research Use Only. Not for use in diagnostic procedures. Not for resale without express authorization.

Product Images For SIRT1 Polyclonal Antibody



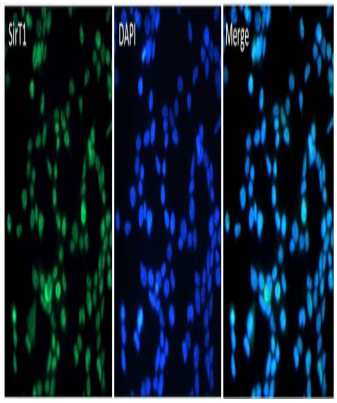
SIRT1 Antibody (PA5-17232) in WB

Western blot was performed using Anti-SIRT1 Polyclonal Antibody (Product # PA5-17232) and a 110 kDa band corresponding to SIRT1 was observed in all cell lysates. Modified whole cell extracts (1% SDS) (30 µg lysate) of HeLa (Lane 1), MCF7 (Lane 2), HEK-293 (Lane 3) and NTERA-2 cl.D1 (Lane 4) were electrophoresed using Novex® NuPAGE® 4-12 % Bis-Tris gel (Product # NP0322BOX). Resolved proteins were then transferred onto a nitrocellulose membrane (Product # IB23001) by iBlot® 2 Dry Blotting System (Product # IB21001). The blot was probed with the primary antibody (1:1000 dilution) and detected by chemiluminescence with Goat anti-Rabbit IgG (Heavy Chain), Superclonal™ Recombinant Secondary Antibody, HRP (Product # A27036, 1:4000 dilution) using the iBright FL 1000 (Product # A32752). Chemiluminescent detection was performed using Novex® ECL Chemiluminescent Substrate Reagent Kit (Product # WP20005).



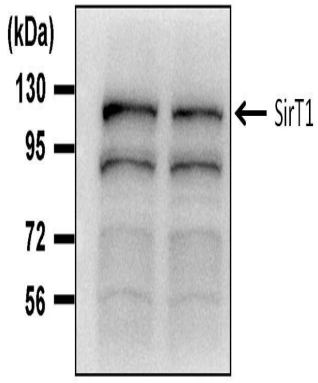
SIRT1 Antibody (PA5-17232) in WB

Western blot analysis of SirT1 was performed by loading 20 µL of HeLa or COS whole cell lysates per well onto a SDS-PAGE gel. Proteins were transferred to a membrane and blocked with 5% non-fat dry milk for 1 hour at room temperature. The membrane was probed with a SirT1 polyclonal antibody (Product # PA5-17232) at a dilution of 1: 1000 overnight at 4°C on a rocking platform, washed in TBST, and probed with a peroxidase-conjugated anti-rabbit IgG secondary antibody for 1 hour at room temperature. Detection was performed using a chemiluminescent substrate.



SIRT1 Antibody (PA5-17232) in ICC/IF

Immunofluorescent analysis of SirT1 (green) HEK293T cells. Cells fixed in 4% formaldehyde were permeabilized and blocked with 1X PBS containing 5% BSA and 0.3% Triton X-100 for 1 hour at room temperature. Cells were probed with a SirT1 polyclonal antibody (Product # PA5-17232) at a dilution of 1:100 overnight at 4°C in 1X PBS containing 1% BSA and 0.3% Triton X-100, washed with 1X PBS, and incubated with a fluorophore-conjugated goat anti-rabbit IgG secondary antibody at a dilution of 1:200 for 1 hour at room temperature. Nuclei (blue) were stained with DAPI. Images were taken on a Leica DM1000 microscope at 40X magnification. Data courtesy of the Innovators Program.



SIRT1 Antibody (PA5-17232) in WB

Western blot analysis of SirT1 was performed by loading 20 µg of THP-1 whole cell lysates per well onto an SDS-PAGE gel. Proteins were transferred to a PVDF membrane and blocked with 5% milk in TBST for 1 hr at room temperature. The membrane was probed with a SirT1 polyclonal antibody (Product # PA5-17232) at a dilution of 1: 500 overnight at °°C, washed in TBST, and probed with a HRP-conjugated goat anti-rabbit IgG at a dilution of 1: 40,000 for 1 hr at room temperature. Detection was performed using ECL substrate. Data courtesy of the Innovators Program.

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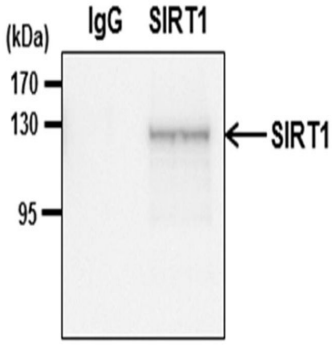
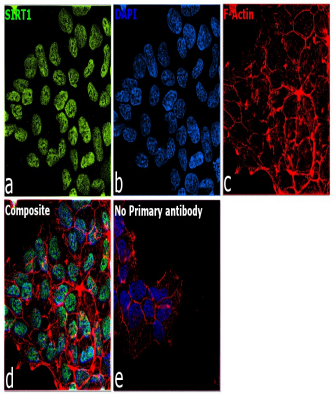


SIRT1 Antibody (PA5-17232) in IP

Immunoprecipitation of SirT1 was performed on HEK293T cells. Antigen-antibody complexes were formed by incubating 500 µg of HEK293T whole cell lysate (in 300 µL volume with 5 µL of a SirT1 polyclonal antibody (Product # PA5-17232) or a rabbit IgG (negative control) overnight at 4°C. The immune complexes were captured on 30 µL of protein G sepharose beads, washed extensively, and eluted with 6X Laemmli buffer. Samples were resolved on an 8% SDS-PAGE gel, transferred to a PVDF membrane, and blocked with 5% milk in TBST for 1 hour at room temperature. The membrane was probed with a SirT1 polyclonal antibody (Product # PA5-17232) at a dilution of 1: 1000 overnight at 4°C, washed in TBST, and probed with a peroxidase-conjugated goat anti-rabbit IgG secondary antibody at a dilution of 1:40,000 for 1 hour at room temperature. Chemiluminescent detection was performed using ECL substrate. Data courtesy of the Innovators Program.



SIRT1 Antibody (PA5-17232) in ICC/IF

Immunofluorescence analysis of SIRT1 was performed using 70% confluent log phase HEK-293 cells. The cells were fixed with 4% paraformaldehyde for 10 minutes, permeabilized with 0.1% Triton™ X-100 for 15 minutes, and blocked with 2% BSA for 1 hour at room temperature. The cells were labeled with SIRT1 Polyclonal Antibody (Product # PA5-17232) at 1:100 dilution in 0.1% BSA, incubated at 4 degree Celsius overnight and then labeled with Donkey anti-Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor Plus 488 (Product # A32790) at a dilution of 1:2000 for 45 minutes at room temperature (Panel a: green). Nuclei (Panel b: blue) were stained with SlowFade® Gold Antifade Mountant with DAPI (Product # S36938). F-actin (Panel c: red) was stained with Rhodamine Phalloidin (Product # R415, 1:300). Panel d represents the merged image showing predominantly nuclear localization. Panel e represents control cells with no primary antibody to assess background. The images were captured at 60X magnification.

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PubMed References For SIRT1 Polyclonal Antibody

1 Western Blot References

Species / Dilution	Summary
	PA5-17232 was used in Western Blotting to identify niacin to be important in the treatment of premature ovarian failure by inhibiting follicular apopotosis under harmful conditions and increases developing follicle numbers upon administration.
Not Applicable / Not Cited	Cellular physiology and biochemistry : international journal of experimental cellular physiology, biochemistry, and pharmacology (2018; 50: 2060) **"Niacin Inhibits Apoptosis and Rescues Premature Ovarian Failure."** Author(s):Wang S,Sun M,Yu L,Wang Y,Yao Y,Wang D PubMed Article URL:http://dx.doi.org/10.1159/000495051

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