Store at -20°C

#9787

Sirtuin Antibody Sampler Kit

☑ 1 Kit
(7 x 20 µl)

Cell Signaling TECHNOLOGY®

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For Research Use Only. Not For Use In Diagnostic Procedures.

Products Included	Product #	Quantity	Mol. Wt.	Isotype
Phospho-SirT1 (Ser47) Antibody	2314	20 µl	120 kDa	Rabbit IgG
SirT1 (D1D7) Rabbit mAb	9475	20 µl	120 kDa	Rabbit IgG
SirT2 (D4O5O) Rabbit mAb	12650	20 µl	39, 43 kDa	Rabbit IgG
SirT3 (D22A3) Rabbit mAb	5490	20 µl	28 kDa	Rabbit IgG
SirT5 (D8C3) Rabbit mAb	8782	20 µl	30 kDa	Rabbit IgG
SirT6 (D8D12) Rabbit mAb	12486	20 µl	42, 36 kDa	Rabbit IgG
SirT7 (D3K5A) Rabbit mAb	5360	20 µl	45 kDa	Rabbit IgG
Anti-rabbit IgG, HRP-linked Antibody	7074	100 µl		Goat

See www.cellsignal.com for individual component applications, species cross-reactivity, dilutions and additional application protocols.

Storage: Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 µg/ml BSA, 50% glycerol and less than 0.02% sodium azide. Store at –20°C. *Do not aliquot the antibodies.*

Recommended Antibody Dilutions:

Western blotting	1:1000
Phospho-SirT1 (Ser47) Antibody 2314	1:2000

Please visit www.cellsignal.com for validation data and a complete listing of recommended companion products.

Description: The Sirtuin Antibody Sampler Kit provides an economical means of evaluating total levels of sirtuin proteins. The kit includes enough antibody to perform two western blot experiments with each primary antibody.

Background: The Silent Information Regulator (SIR2) family of genes is a highly conserved group of genes that encode nicotinamide adenine dinucleotide (NAD)-dependent protein deacetylases, also known as Class III histone deacetylases. The best characterized of these genes is *Saccharomyces cerevisiae Sir2*, which is involved in silencing of mating type loci, telomere maintenance, DNA damage response and cell aging (1). SirT1, the mammalian ortholog of Sir2, is a nuclear protein implicated in the regulation of apoptosis, cellular senescence, endocrine signaling, glucose homeostasis, aging, and longevity. SirT2, one of several mammalian Sir2 homologs, deacetylates α-tubulin on Lys40 and histone H4 on Lys16, and is implicated in cytoskeletal regulation and progression through mitosis (2,3). SirT2 protein is mainly cytoplasmic and is associated with microtubules and the HDAC6 tubulin deacetylase (2).

SirT3 exists in human cells in two forms, including a full-length, nuclear (44 kDa) protein and a processed (28 kDa) protein found exclusively in the mitochondria (4-6). Full-length SirT3 protein is processed in the mitochondrial matrix by mitochondrial matrix processing peptidase (MMP) (5). Both full-length and processed SirT3 are active enzymes that deacetylate histone H3 at Lys9 and histone H4 at Lys16 *in vitro* (4). SirT3 also deacetylates Lys642 of acetyl-CoA synthetase 2 (AceCS2) and activates AceCS2 activity in the mitochondria (7).

SirT5 is localized to the mitochondria and has been implicated in the regulation of cell metabolism (8,9). Nuclear SirT6 is a chromatin-associated protein that promotes normal maintenance of genome integrity as mediated by the base excision repair (BER) pathway (10-12). Mammalian SirT7 is localized to the nucleolus and is prominently expressed in hematopoietic cells, especially myeloid progenitor cells (13). SirT7 is recruited to chromatin by sequence-specific DNA binding transcription factors such as Elk-4, where it facilitates transcriptional repression through deacetylation of histone H3 at Lys18 (14).

Specificity/Sensitivity: Each antibody in the Sirtuin Antibody Sampler Kit recognizes endogenous levels of its specific target. Activation state antibodies detect their intended targets only when phosphorylated at the indicated site.

Source/Purification: Monoclonal antibodies are produced by immunizing animals with synthetic peptides corresponding to residues surrounding Phe297 of human SirT1 protein, Pro205 of human SirT2, or Val130 of mouse SirT3 protein, and with recombinant proteins specific to full-length human SirT5 protein, full-length mouse SirT6, or the amino terminus of human SirT7 protein. Polyclonal antibodies are produced by immunizing animals with a synthetic phosphopeptide corresponding to residues surrounding Ser47 of human SirT1. Polyclonal antibodies are purified by Protein A and peptide affinity chromatography.

Background References:

(1) Guarente, L. (1999) *Nat Genet* 23, 281-5.
(2) North, B.J. et al. (2003) *Mol Cell* 11, 437-44.
(3) Vaquero, A. et al. (2006) *Genes Dev* 20, 1256-61.
(4) Scher, M.B. et al. (2007) *Genes Dev* 21, 920-8.
(5) Schwer, B. et al. (2002) *J Cell Biol* 158, 647-57.
(6) Onyango, P. et al. (2002) *Proc Natl Acad Sci USA* 99, 13653-8.
(7) Schwer, B. et al. (2006) *Proc Natl Acad Sci USA* 103, 10224-9.
(8) Newman, J.C. et al. (2012) *J Biol Chem* 287, 42436-43.
(9) He, W. et al. (2012) *Trends Endocrinol Metab* 23, 467-76.
(10) Mostoslavsky, R. et al. (2006) *Cell* 124, 315-29.
(11) Liszt, G. et al. (2005) *J Biol Chem* 280, 21313-20.
(12) Michishita, E. et al. (2005) *Mol Biol Cell* 16, 4623-35.
(13) Voelter-Mahlknecht, S. et al. (2006) *Int J Oncol* 28, 899-908.
(14) Barber, M.F. et al. (2012) *Nature* 487, 114-8.

Applications Key: **W**—Western **IP**—Immunoprecipitation **IHC**—Immunohistochemistry **ChIP**—Chromatin Immunoprecipitation **IF**—Immunofluorescence **F**—Flow cytometry **E-P**—ELISA-Peptide

Species Cross-Reactivity Key: **H**—human **M**—mouse **R**—rat **Hm**—hamster **Mk**—monkey **Mi**—mink **C**—chicken **Dm**—D. melanogaster **X**—Xenopus **Z**—zebrafish **B**—bovine **Dg**—dog **Pg**—pig **Sc**—S. cerevisiae **All**—all species expected Species enclosed in parentheses are predicted to react based on 100% homology.

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Western Immunoblotting Protocol

For western blots, incubate membrane with diluted primary antibody in either 5% w/v BSA or nonfat dry milk, 1X TBS, 0.1% Tween® 20 at 4°C with gentle shaking, overnight.
NOTE: Please refer to primary antibody datasheet or product webpage for recommended primary antibody dilution buffer and recommended antibody dilution.

A. Solutions and Reagents

NOTE: Prepare solutions with reverse osmosis deionized (RODI) or equivalent grade water.

1. **20X Phosphate Buffered Saline (PBS):** (#9808) To prepare 1 L 1X PBS: add 50 ml 20X PBS to 950 ml dH_2O, mix.
2. **10X Tris Buffered Saline (TBS):** (#12498) To prepare 1 L 1X TBS: add 100 ml 10X to 900 ml dH_2O, mix.
3. **1X SDS Sample Buffer:** Blue Loading Pack (#7722) or Red Loading Pack (#7723) Prepare fresh 3X reducing loading buffer by adding 1/10 volume 30X DTT to 1 volume of 3X SDS loading buffer. Dilute to 1X with dH_2O.
4. **10X Tris-Glycine SDS Running Buffer:** (#4050) To prepare 1 L 1X running buffer: add 100 ml 10X running buffer to 900 ml dH_2O, mix.
5. **10X Tris-Glycine Transfer Buffer:** (#12539) To prepare 1 L 1X transfer buffer: add 100 ml 10X transfer buffer to 200 ml methanol + 700 ml dH_2O, mix.
6. **10X Tris Buffered Saline with Tween® 20 (TBST):** (#9997) To prepare 1 L 1X TBST: add 100 ml 10X TBST to 900 ml dH_2O, mix.
7. **Nonfat Dry Milk:** (#9999)
8. **Blocking Buffer:** 1X TBST with 5% w/v nonfat dry milk; for 150 ml, add 7.5 g nonfat dry milk to 150 ml 1X TBST and mix well.
9. **Wash Buffer:** (#9997) 1X TBST
10. **Bovine Serum Albumin (BSA):** (#9998)
11. **Primary Antibody Dilution Buffer:** 1X TBST with 5% BSA or 5% nonfat dry milk as indicated on primary antibody datasheet; for 20 ml, add 1.0 g BSA or nonfat dry milk to 20 ml 1X TBST and mix well.
12. **Biotinylated Protein Ladder Detection Pack:** (#7727)
13. **Prestained Protein Marker, Broad Range (Premixed Format):** (#7720)
14. **Blotting Membrane and Paper:** (#12369) This protocol has been optimized for nitrocellulose membranes. Pore size 0.2 µm is generally recommended.
15. **Secondary Antibody Conjugated to HRP:** anti-rabbit (#7074); anti-mouse (#7076)
16. **Detection Reagent:** LumiGLO® chemiluminescent reagent and peroxide (#7003) or SignalFire™ ECL Reagent (#6883)

B. Protein Blotting

A general protocol for sample preparation.

1. Treat cells by adding fresh media containing regulator for desired time.
2. Aspirate media from cultures; wash cells with 1X PBS; aspirate.
3. Lyse cells by adding 1X SDS sample buffer (100 µl per well of 6-well plate or 500 µl for a 10 cm diameter plate). Immediately scrape the cells off the plate and transfer the extract to a microcentrifuge tube. Keep on ice.
4. Sonicate for 10–15 sec to complete cell lysis and shear DNA (to reduce sample viscosity).
5. Heat a 20 µl sample to 95–100°C for 5 min; cool on ice.
6. Microcentrifuge for 5 min.
7. Load 20 µl onto SDS-PAGE gel (10 cm x 10 cm). **NOTE:** Loading of prestained molecular weight markers (#7720, 10 µl/lane) to verify electrotransfer and biotinylated protein ladder (#7727, 10 µl/lane) to determine molecular weights are recommended.
8. Electrotransfer to nitrocellulose membrane (#12369).

C. Membrane Blocking and Antibody Incubations

NOTE: Volumes are for 10 cm x 10 cm (100 cm^2) of membrane; for different sized membranes, adjust volumes accordingly.

I. Membrane Blocking

1. (Optional) After transfer, wash nitrocellulose membrane with 25 ml TBS for 5 min at room temperature.
2. Incubate membrane in 25 ml of blocking buffer for 1 hr at room temperature.
3. Wash three times for 5 min each with 15 ml of TBST.

II. Primary Antibody Incubation

1. Incubate membrane and primary antibody (at the appropriate dilution and diluent as recommended in the product datasheet) in 10 ml primary antibody dilution buffer with gentle agitation overnight at 4°C.
2. Wash three times for 5 min each with 15 ml of TBST.
3. Incubate membrane with the species appropriate HRP-conjugated secondary antibody (#7074 or #7076 at 1:2000) and anti-biotin, HRP-linked Antibody (#7075 at 1:1000–1:3000) to detect biotinylated protein markers in 10 ml of blocking buffer with gentle agitation for 1 hr at room temperature.
4. Wash three times for 5 min each with 15 ml of TBST.
5. Proceed with detection (Section D).

D. Detection of Proteins

1. Incubate membrane with 10 ml LumiGLO® (0.5 ml 20X LumiGLO® #7003, 0.5 ml 20X peroxide, and 9.0 ml purified water) or 10 ml SignalFire™ #6883 (5 ml Reagent A, 5 ml Reagent B) with gentle agitation for 1 min at room temperature.
2. Drain membrane of excess developing solution (do not let dry), wrap in plastic wrap and expose to x-ray film. An initial 10 sec exposure should indicate the proper exposure time. **NOTE:** Due to the kinetics of the detection reaction, signal is most intense immediately following incubation and declines over the following 2 hr.