

Human Sirtuin 1/SIRT1 Antibody

Antigen Affinity-purified Polyclonal Sheep IgG Catalog Number: AF7714

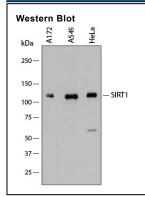
DESCRIPTION		
Species Reactivity	Human	
Specificity	Detects human Sirtuin 1/SIRT1 in direct ELISAs and Western blots. In direct ELISAs, less than 1% cross-reactivity with recombinant human SIRT2 is observed.	
Source	Polyclonal Sheep IgG	
Purification	Antigen Affinity-purified	
Immunogen	E. coli-derived recombinant human Sirtuin 1/SIRT1 Ala2-Ser747 Accession # Q96EB6	
Formulation	Lyophilized from a 0.2 µm filtered solution in PBS with Trehalose. See Certificate of Analysis for details. *Small pack size (-SP) is supplied either lyophilized or as a 0.2 µm filtered solution in PBS.	

APPLICATIONS

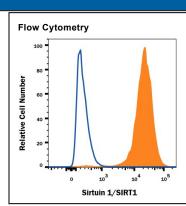
Please Note: Optimal dilutions should be determined by each laboratory for each application. General Protocols are available in the Technical Information section on our website.

	Recommended Concentration	Sample	
Western Blot	0.2 μg/mL	See Below	
Flow Cytometry	0.25 μg/10 ⁶ cells	See Below	
CyTOF-ready	Ready to be labeled using established conjugation methods. No BSA or other carrier proteins that could interfere with conjugation.		
Knockout Validated	Sirtuin 1/SIRT1 is specifically detected in HeLa human cervical epithelial carcinoma parental cell line but is not detectable in Sirtuin 1/SIRT1 knockout HeLa cell line.		

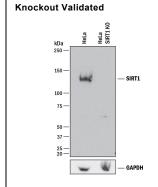
DATA



Detection of Human Sirtuin 1/SIRT1 by Western Blot, Western blot shows lysates of A172 human glioblastoma cell line, A549 human lung carcinoma cell line, and HeLa human cervical epithelial carcinoma cell line. PVDF membrane was probed with 0.2 μ g/mL of Sheep Anti-Human Sirtuin 1/SIRT1 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF7714) followed by HRPconjugated Anti-Sheep IgG Secondary Antibody (Catalog # HAF016). A specific band was detected for Sirtuin 1/SIRT1 at approximately 120 kDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.



Detection of Sirtuin 1/SIRT1 in HepG2 Human Cell Line by Flow Cytometry. HepG2 human hepatocellular carcinoma cell line was stained with Sheep Anti-Human Sirtuin 1/SIRT1 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF7714, filled histogram) or isotype control antibody (Catalog # 5-001-A, open histogram), followed by Allophycocyanin-conjugated Anti-Sheep IgG Secondary Antibody (Catalog # F0127). To facilitate intracellular staining, cells were fixed and permeabilized with FlowX FoXP3 Fixation & Permeabilization Buffer Kit (Catalog # FC012). View our protocol for Staining Intracellular Molecules.



Western Blot Shows Human Sirtuin 1/SIRT1 Specificity by Using Knockout Cell Line. Western blot shows lysates of HeLa human cervical epithelial carcinoma parental cell line and SIRT1 knockout HeLa cell line (KO). PVDF membrane was probed with 0.2 µg/mL of Sheep Anti-Human Sirtuin 1/SIRT1 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF7714) followed by HRP-conjugated Anti-Sheep IgG Secondary Antibody (Catalog # HAF016). A specific band was detected for Sirtuin 1/SIRT1 at approximately 120 kDa (as indicated) in the parental HeLa cell line, but is not detectable in knockout HeLa cell line. GAPDH (Catalog # AF5718) is shown as a loading control. This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.

Rev. 2/6/2018 Page 1 of 2





Human Sirtuin 1/SIRT1 Antibody

Antigen Affinity-purified Polyclonal Sheep IgG Catalog Number: AF7714

PREPARATION AND STORAGE		
Reconstitution	Sterile PBS to a final concentration of 0.2 mg/mL.	
Shipping	The product is shipped at ambient temperature. Upon receipt, store it immediately at the temperature recommended below. *Small pack size (-SP) is shipped with polar packs. Upon receipt, store it immediately at -20 to -70 °C	
Stability & Storage	Use a manual defrost freezer and avoid repeated freeze-thaw cycles. 12 months from date of receipt, -20 to -70 °C as supplied. 1 month, 2 to 8 °C under sterile conditions after reconstitution. 6 months, -20 to -70 °C under sterile conditions after reconstitution.	

BACKGROUND

SIRT1 (SIR2-like protein 1; also NAD-dependent protein deacetylase sirtuin-1 and hSIR2) is a class I member of the sirtuin family of enzymes. Although its predicted MW is 81 kDa, it runs anomalously at 110-120 kDa in SDS-PAGE. It is a widely expressed nuclear protein that participates in the deacetylation of multiple proteins, including p300, p53, LKB1 and histone H1. Functionally, this has the effect of promoting heterochromatin formation, cell survival and resistance to oxidative stress. Metabolically, SIRT1 induces insulin secretion, inhibits glycolysis and suppresses fatty acid synthesis. Human SIRT1 is 747 amino acids (aa) in length. It possesses two NLS's (aa 32-39 and 223-230), an NES (aa 138-145), and a sertuin-type deacetylase domain (aa 241-495) that contains an NAD and Zn binding motif. There are at least 12 utilized Ser/Thr phosphorylation sites, plus two nitrosylated Cys and one acetylated Ala. There are also four potential isoform variants. One is 95 kDa in size and shows a deletion of aa 454-639, a second is 17 kDa in size and contains a 16 aa substitution for aa 149-747, and a third contains an alternative start site at Met296. SIRT1 is also known to undergo proteolysis by cathepsin B at Val533Ser534, generating a fourth, C-terminally truncated 75 kDa isoform. Full-length SIRT1 is suggested to form trimers, while the 17 kDa isoform appears to form dimers. Over aa 2-747, human and mouse SIRT1 share 86% aa sequence identity.



