Purified anti-Histone H3.1 Phospho (Ser28) Antibody

Catalog# / Size	687602 / 100 µg
Clone	5D10D4
Regulatory Status	RUO
Other Names	Histone-H3
Isotype	Rat IgG2a, κ
Description	Histone proteins are classified into core histones (H2A, H2B, H3, H4) and linker histones (H1, H5). Core histones form an octamer, which contains two H2A-H2B dimers and one H3-H4 tetramer. Core histones are predominantly globular except for the unstructured N-terminal tails. Posttranslational modifications, such as acetylation, methylation, phosphorylation, ubiquitination, SUMOylation and ADP-ribosylation occur in histone tails. Histone modifications induce changes of chromatin structure and thereby affect the accessibility of transcription factors, nuclear proteins and enzymes to genomic DNA, resulting in gene activation or repression. It is known that histone modifications play critical roles in DNA repair, DNA replication, transcription regulation, alternative splicing and chromosome condensation and some diseases including autoimmune diseases and cancers.

Product Details

Verified Reactivity	Human
Antibody Type	Monoclonal
Host Species	Rat
Immunogen	Histone H3.1 S28ph peptide (21-39)(ATKAARK(phS)APATGGVKKPH), Freund's complete adjuvant.
Formulation	Phosphate-buffered solution, pH 7.2, containing 0.09% sodium azide.
Preparation	The antibody was purified by affinity chromatography.
Concentration	0.5 mg/mL
Storage & Handling	The antibody solution should be stored undiluted between 2°C and 8°C.
Application	ICFC - Quality tested WB, ChIP - Verified Direct ELISA, ICC - Reported in the literature, not verified in house
Recommended Usage	Each lot of this antibody is quality control tested by intracellular immunofluorescent staining with flow cytometric analysis. For flow cytometric staining, the suggested use of this reagent is ≤ 0.25 µg per million cells in 100 µL volume. For ChIP applications, the suggested dilution for ChIP application is 1:50-1:100 by volume. For Western blotting, the suggested use of this reagent is 0.5 - 2.0 µg/mL. It is recommended that the reagent be titrated for optimal performance for each application.
RRID	AB_2616945 (BioLegend Cat. No. 687602)

Antigen Details

Structure	H3 is part of the nucleosome, comprised of an octameric complex with H2A, H2B, and H4 proteins.
Distribution	Nucleus.
Function	H3 is a core component of the nucleosome that serves to wrap and compact DNA into chromatin. Therefore, histones limit the accessibility of DNA by providing mechanisms for transcription regulation, DNA repair and replication and chromosomal stability.

Interaction	Two molecules of H3 form a heterotetramer with two molecules of H4.
Biology Area	Cell Biology, Cell Cycle/DNA Replication, Cell Proliferation and Viability
Molecular Family	Phospho-Proteins
Antigen References	1. Choi HS, *et al.* 2005. *J. Biol. Chem.* 280:13545. 2. Goto H, *et al.* 2002. *Genes Cells* 7:11. 3. Garcia BA, *et al.* 2005. *Biochemistry* 44:13202. 4. Yoshimi T, *et al.* 2013. *Monoclon. Immunodiagn. Immunother.* 32:119.
Gene ID	8350

Related Protocols

Surface and Intracellular Cytokine Staining for Flow Cytometry - Video

BioLegend's Tools for Chromatin Immunoprecipitation (ChIP) Assays - Video

Chromatin Immunoprecipitation (ChIP) Assay Protocol

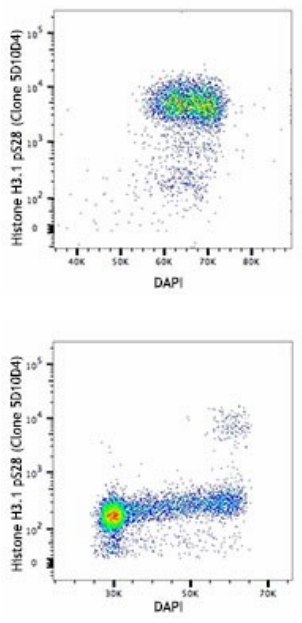
Western Blotting Protocol

Intracellular Flow Cytometry Staining Protocol

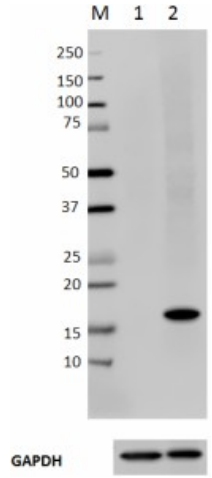
Other Formats

Purified anti-Histone H3.1 Phospho (Ser28), Alexa Fluor® 647 anti-Histone H3.1 Phospho (Ser28)

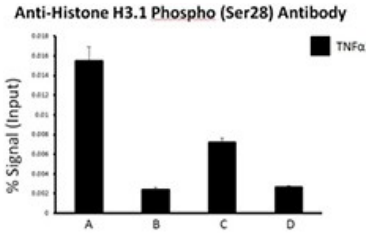
Product Data



HeLa cells were stimulated with (top) or without (bottom) nocodozole for 24 hours, then fixed and permeabilized with cold 70% ethanol, then intracellularly stained with DAPI and purified anti-Histone H3.1 Phospho (Ser28) antibody (clone 5D10D4), followed by anti-rat IgG PE.



Total lysates (15 µg protein) from HeLa (lane 1) and HeLa cells treated with Nocodazole for 24 hours (lane 2) were resolved by electrophoresis (4-12% Bis-Tris gel), transferred to nitrocellulose, and probed with 1 µg/mL Purified anti-Histone H3.1 Phospho (ser28) Antibody, clone 5D10D4 (upper), or 1:2000 diluted anti-GAPDH Antibody (lower). Proteins were visualized by chemiluminescence detection using a 1:3000 diluted goat anti-Rat-IgG secondary antibody conjugated to HRP for the anti-Histone H3.1 Phospho (ser28) Antibody, or 1:3000 diluted donkey anti-Rabbit-IgG secondary antibody conjugated to HRP for anti-GAPDH Antibody. Lane M: Molecular weight ladder.



Chromatin Immunoprecipitation (ChIP) was performed using commercial Protein-G coated 96 well high-throughput ChIP assay kit by loading 3µg of cross-linked chromatin samples from HeLa cells treated with Nocodazole with either A) 1:50 dilution of Go-ChIP-Grade™ Purified anti-Histone H3.1 Phospho (Ser28) Antibody (Clone 5D10D4), B) equal amount of Purified Rat IgG2a, κ Isotype Control Antibody, or C) competitor's ChIP-grade Purified anti-Histone H3.1 Phospho (Ser28) Antibody and D) equal amount of matched Isotype Control Antibody as recommended by the manufacturer. The enriched DNA was purified and quantified by real-time qPCR using primers targeting human TNFα gene region. The amount of immunoprecipitated DNA in each sample is represented as signal relative to the 5% of total amount of input chromatin.

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