Purified anti-Histone H3-Phosphorylated (Ser28) Antibody

Catalog# / Size	641001 / 25 µg 641002 / 100 µg
Clone	HTA28
Regulatory Status	RUO
Other Names	Histone-H3
Isotype	Rat IgG2a, κ
Description	H3 is a core component of the nucleosome that serves to wrap and compact DNA into chromatin. Histones therefore, limit the accessibility of DNA, providing mechanisms for transcription regulation, DNA repair and replication and chromosomal stability. During mitosis, H3 is phosphorylated at serine 28.This phosphorylation coincides with chromosome condensation initiated at prophase and disappears at late anaphase. H3 has been demonstrated to be phosphorylated by the action of MLTK-α (mixed linage kinase-like mitogen activated protein triple kinase α) in response to ultraviolet B light and epidermal growth factor, as well as Aurora-B during mitosis.

Product Details

Verified Reactivity	Human
Antibody Type	Monoclonal
Host Species	Rat
Immunogen	Synthetic peptide conjugated to KLH, corresponding to amino acids 23-35 of human histone H3.
Formulation	This antibody is provided in phosphate-buffered solution, pH 7.2, containing 0.09% sodium azide. Final antibody concentration is 0.5 mg/ml.
Preparation	The antibody was purified by affinity chromatography.
Concentration	0.5 mg/ml
Storage & Handling	Upon receipt, store between 2°C and 8°C.
Application	WB - *Quality tested* CyTOF®, ICC - *Verified* IP, ICFC - *Reported in the literature, not verified in house*
Recommended Usage	Each lot of this antibody is quality control tested by Western blotting. For Western blotting, the suggested use of this reagent is 0.5 - 1.0 µg per ml. It is recommended that the reagent be titrated for optimal performance for each application.
Application Notes	This clone is not recommended for ChIP (Chromatin Immunoprecipitation) assays (as determined by in-house testing).

Application References

1. Hirata A, et al. 2004. *J. Histochem. Cytochem.* 52:1503.
2. Goto H, et al. 1999. *J. Biol. Chem.* 274:25543.
3. Ozawa K. 2008. *Cytometry A* 73:517.
4. Goode NJ, *et al.* 2014. *PLoS Genet.* 10:1004323. PubMed

Product Citations

1. Hutton C, *et al.* 2021. Cancer Cell. 39:1227. PubMed
2. de Vargas Roditi L, *et al.* 2022. Cell Rep Med. 3:100604. PubMed
3. Lun XK *et al.* 2019. Mol Cell. 74(5):1086-1102 . PubMed
4. Damond N, *et al.* 2019. Cell Metab. 29:755. PubMed
5. Baran N, *et al.* 2022. Nat Commun. 13:2801. PubMed
6. Tognetti M, *et al.* 2021. Cell Systems. 12(5):401-418.e12. PubMed
7. Godde N, *et al.* 2014. PLoS Genet. 10:1004323. PubMed
8. Bouchard G, *et al.* 2022. Cancer Res. 82:648. PubMed

RRID	AB_1227660 (BioLegend Cat. No. 641001) AB_1227659 (BioLegend Cat. No. 641002)

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. Histones therefore, limit the accessibility of DNA, 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, DNA Repair/Replication, Transcription Factors
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.
Regulation	H3 is regulated by acetylation, methylation, citrullination, phosphorylation, and ubiquitination.
Gene ID	8290

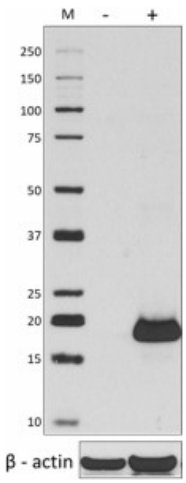
Related Protocols

Western Blotting Protocol

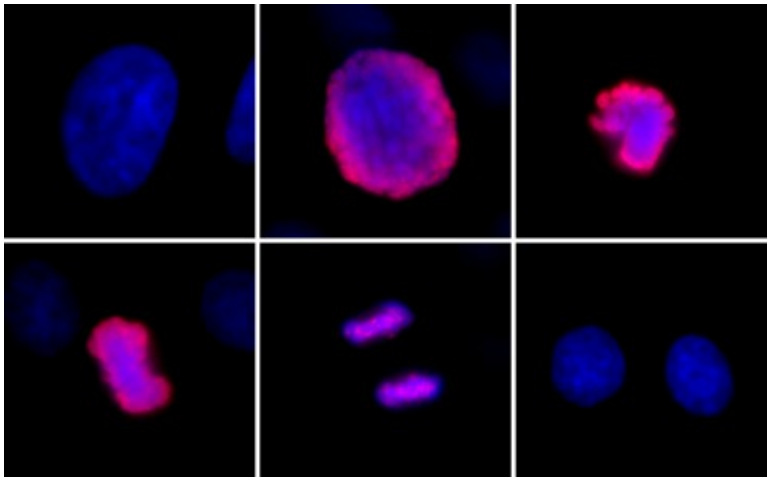
Other Formats

Purified anti-Histone H3-Phosphorylated (Ser28), Alexa Fluor® 488 anti-Histone H3-Phosphorylated (Ser28), Alexa Fluor® 647 anti-Histone H3-Phosphorylated (Ser28), Purified anti-Histone H3-Phosphorylated (Ser28) (Maxpar® Ready), PE anti-Histone H3-Phosphorylated (Ser28), PE/Cyanine7 anti-Histone H3-Phosphorylated (Ser28), PerCP/Cyanine5.5 anti-Histone H3-Phosphorylated (Ser28), Alexa Fluor® 594 anti-Histone H3-Phosphorylated (Ser28), Direct-Blot™ HRP anti-Histone H3 Phospho (Ser28)

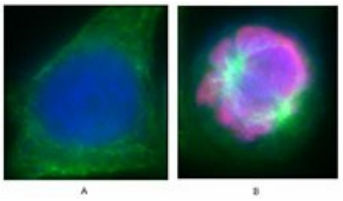
Product Data



Whole cell extracts (15 µg protein) from untreated (-) or nocodazole-treated (+, 300 ng/mL overnight) HeLa cells were resolved on a 4-12% Bis-Tris gel, transferred to a nitrocellulose membrane and probed with 1.0 µg/mL (1:500 dilution) of Purified anti-Histone H3 Phospho (Ser28) Antibody, clone HTA28, overnight at 4°C. Proteins were visualized by chemiluminescence detection using HRP goat anti-mouse IgG Antibody (Cat. No. 405306) at a 1:3000 dilution. Direct-Blot™ anti-β-Actin was used as a loading control at a 1:25000 dilution. Lane M: Molecular Weight marker.



HeLa cells were fixed with 4% paraformaldehyde (PFA) for 15 minutes, permeabilized with 0.5% Triton X-100 for 3 minutes, and blocked with 5% FBS for 60 minutes. Then the cells were intracellularly stained with 1:2500 diluted (0.2 µg/ml) Histone H3-Phosphorylated (Ser28) Antibody (clone HTA28, lower) overnight at 4 degrees followed by Alexa Fluor® 594 (red) conjugated goat anti-rat IgG (Cat. No. 405422) incubation for one hour at room temperature. Nuclei were counterstained with DAPI (blue). The image was captured with a 60X objective. The images displayed HeLa cells during cell cycle progression.



Untreated HeLa (Panel A) and overnight nocodazoled-treated HeLa (Panel B) were stained with purified rat monoclonal antibody against phospho-H3 (Ser28) (clone HTA28), followed by Alexa Fluor® 488 anti-alpha-tubulin, DyLight™ 594 goat anti-rat-IgG and DAPI.

BioLegend Inc., 8999 BioLegend Way, San Diego, CA 92121 www.biolegend.com
Toll-Free Phone: 1-877-Bio-Legend (246-5343) Phone: (858) 768-5800 Fax: (877) 455-9587