

Datasheet for 600-401-I84**Histone H3 K27me3/phospho S28 Antibody****Overview**

Description:	Anti-Histone H3 [Trimethyl Lys27, p Ser28] (RABBIT) Antibody - 600-401-I84
Item No.:	600-401-I84
Size:	50 µg
Applications:	ChIP, Dot Blot, IF, Multiplex, WB
Reactivity:	Human, Mouse, C. elegans
Host Species:	Rabbit

Product Details

Background:	Chromatin is the arrangement of DNA and proteins in which chromosomes are formed. Correspondingly, chromatin is formed from nucleosomes, which are comprised of a set of four histone proteins (H2A, H2B, H3, H4) wrapped with DNA. Chromatin is a very dynamic structure in which numerous post-translational modifications work together to activate or repress the availability of DNA to be copied, transcribed, or repaired. These marks decide which DNA will be open and commonly active (euchromatin) or tightly wound to prevent access and activation (heterochromatin). Common histone modifications include methylation of lysine and arginine, acetylation of lysine, phosphorylation of threonine and serine, and sumoylation, biotinylation, and ubiquitylation of lysine. Specifically, trimethylation of K27 is associated with gene silencing, whereas pS28 is associated with mitosis and immediate early genes. Anti-Histone H3 are ideal for researchers interested in Chromatin Modifiers, Chromatin Research, Histones and Modified Histones, and Epigenetics research.
Synonyms:	rabbit anti-Histone H3 trimethyl Lys18 pS28 antibody, H3.3B, H3pS28K18Me3, H3 histone, family 3A, H3.3AH3F3H3F3B, histone H3.3, MGC87783, MGC87782, H3 K27me3/pS28
Host Species:	Rabbit
Clonality:	Polyclonal
Format:	IgG

Target Details

Gene Name:	HIST2H3C
Reactivity:	Human, Mouse, C. elegans

PTM Specificity:	Dual Modification
Immunogen Type:	Conjugated Peptide
Immunogen:	Histone H3 [Trimethyl Lys27, p Ser28] affinity purified antibody was prepared from whole rabbit serum produced by repeated immunizations with synthetic trimethylated/phosphorylated peptides surrounding Lysine 27 and Serine 28 of human Histone H3.
Purity/Specificity:	Anti-Histone H3 [Trimethyl Lys27, p Ser28] was affinity purified from monospecific antiserum by immunoaffinity chromatography. This antibody reacts with human Histone H3. A BLAST analysis was used to suggest cross-reactivity with Human, mouse, and C. elegans. Predicted to react with many species including rat, chicken, Xenopus, Drosophila, and plant based on 100% sequence homology. Cross-reactivity with Histone H3 from other sources has not been determined.
Relevant Links:	• UniProtKB - Q71DI3 • NCBI - NP_001005464 • GeneID - 126961

Application Details

Tested Applications:	ChIP, Dot Blot, IF, Multiplex, WB
Application Note:	Anti-Histone H3 [Trimethyl Lys27, p Ser28] antibody is tested for Western Blot, Chromatin Immunoprecipitation, Dot Blot, and Immunocytochemistry/Immunofluorescence. Specific conditions for reactivity should be optimized by the end user. Expect a band approximately ~15.4 kDa corresponding to Histone H3 protein by Western Blotting in HeLa histone prep lysate or the appropriate cell lysate or extract. Epi-Plus™ antibody production in collaboration with Novus Biologicals.
Assay Dilutions:	All assays should be optimized by the user. Recommended dilutions (if any) may be listed below.
ChIP:	2-5µg/million cells
IF:	1:2000
IHC:	1:2000
WB:	1 µg/mL

Formulation

Physical State:	Liquid (sterile filtered)
Concentration:	0.66mg/mL by UV absorbance at 280 nm
Buffer:	0.02 M Potassium Phosphate, 0.15 M Sodium Chloride, pH 7.2

Preservative: 0.05% (w/v) Sodium Azide

Stabilizer: 30% Glycerol

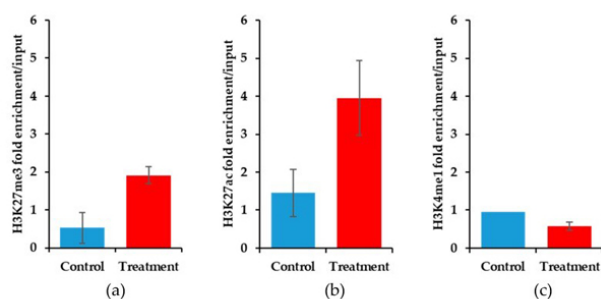
Shipping & Handling

Shipping Condition: Dry Ice

Storage Condition: Store vial at -20° C prior to opening. Aliquot contents and freeze at -20° C or below for extended storage. Avoid cycles of freezing and thawing. Centrifuge product if not completely clear after standing at room temperature. This product is stable for several weeks at 4° C as an undiluted liquid. Dilute only prior to immediate use.

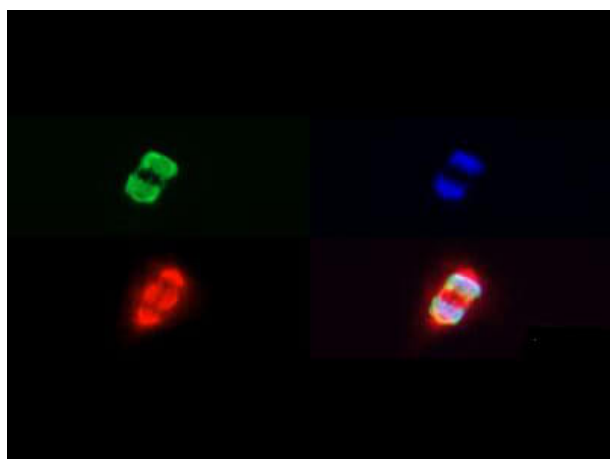
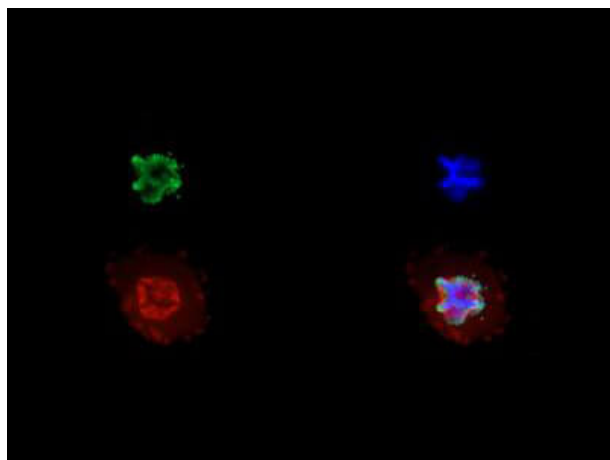
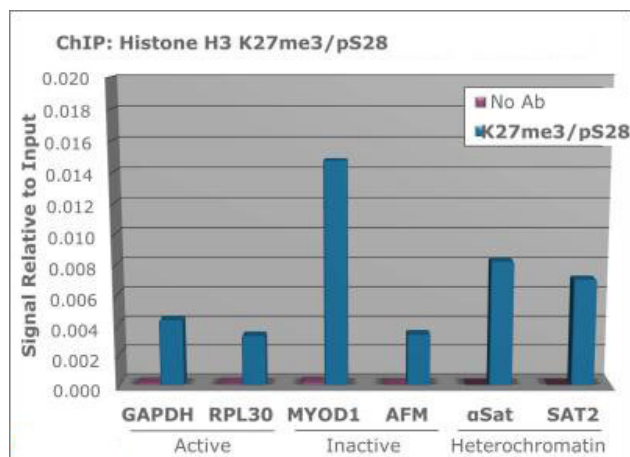
Expiration: Expiration date is one (1) year from date of receipt.

Images



ChIP

Epigenetic histone marks differ between control and YM155 treated mouse synovial sarcoma at the Birc5 promoter. (a) ChIP-qPCR for H3K27me3 shows elevated levels at the Birc5 promoter in the YM155 treated samples (n = 5) compared to controls (n = 3), error bars are SEM (p = 0.045). (b) ChIP-qPCR for H3K27ac shows elevated levels at the Birc5 promoter in the YM155 treated samples (n = 5) compared to controls (n = 3), error bars are SEM (p = 0.14). (c) ChIP-qPCR for H3K4me1 shows equally low levels at the Birc5 promoter between the YM155 treated samples (n = 5) compared to controls (n = 2), error bars for treated samples are SEM (p = 0.13). The following antibodies were used for immunoprecipitation. anti-H3K27me3 (Rockland, 600-401-I84), anti-H3K27ac (Rockland, 600-401-K00), anti-H3K4me1 (Rockland, 600-401-I61). Fig 7. PMID: 30909651



ChIP

Chromatin Immunoprecipitation Rabbit Anti-Histone H3 [Trimethyl Lys27, p Ser28] Antibody. Chromatin from one million formaldehyde cross-linked HeLa cells was used with 2ug of Anti-Histone H3 K27me3pS28 and 20ul of magnetic IgG beads per immunoprecipitation. A no antibody (No Ab) control was also used. Immunoprecipitated DNA was quantified using quantitative real-time PCR and SYBR green dye, then normalized to the non-precipitated input chromatin, which is equal to one.

Immunofluorescence Microscopy

Immunofluorescence of Rabbit Anti-Histone H3 [p Ser28, Trimethyl Lys27] Antibody. Tissue: HeLa cells. Fixation: 0.5% PFA. Antigen retrieval: Not required. Primary antibody: Histone H3 [p Ser28, Trimethyl Lys27] antibody at a 1:2000 dilution for 1 h at RT. Secondary antibody: Dylight 488 secondary antibody at 1:10,000 for 45 min at RT. Localization: Histone H3 [p Ser28, Trimethyl Lys27] is nuclear and chromosomal. Staining: Histone H3 [p Ser28, Trimethyl Lys27] is expressed in green, nuclei and alpha-tubulin are counterstained with DAPI (blue) and Dylight 550 (red).

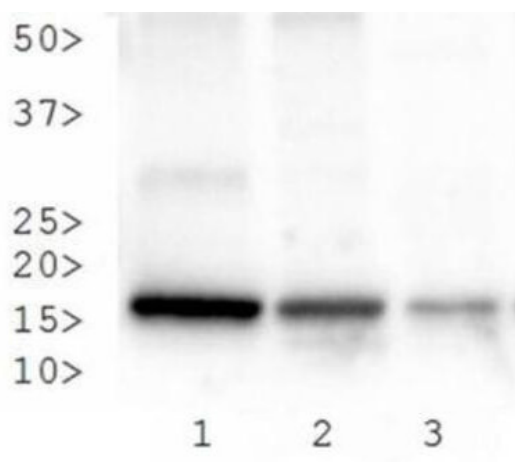
Immunofluorescence Microscopy

Immunofluorescence of Rabbit Anti-Histone H3 [p Ser28, Trimethyl Lys27] Antibody. Tissue: HeLa cells. Fixation: 0.5% PFA. Antigen retrieval: Not required. Primary antibody: Histone H3 [p Ser28, Trimethyl Lys27] antibody at a 1:2000 dilution for 1 h at RT. Secondary antibody: Dylight 488 secondary antibody at 1:10,000 for 45 min at RT. Localization: Histone H3 [p Ser28, Trimethyl Lys27] is nuclear and chromosomal. Staining: Histone H3 [p Ser28, Trimethyl Lys27] is expressed in green, nuclei and alpha-tubulin are counterstained with DAPI (blue) and Dylight 550 (red).



Dot Blot

Dot Blot of Rabbit Histone H3 [p Ser28, Trimethyl Lys27] Antibody. Lane 1: pS28/K27 unmodified. Lane 2: pS28 N-Term. Lane 3: p28 C-term. Lane 4: K27Me3. Lane 5: pS28/K27Me3. Load: 1, 10, and 100 picomoles of peptide. Primary antibody: Histone H3 [p Ser28, Trimethyl Lys27] antibody at 1 µg/ml for 45 min at 4°C. Secondary antibody: Dylight™488 rabbit secondary antibody at 1:10,000 for 45 min at RT. Block: 5% BLOTTO overnight at 4°C.



Western Blot

Western Blot of Rabbit Anti-Histone H3 [Trimethyl Lys27, p Ser28] Antibody. Lane 1: HeLa histone prep. Lane 2: NIH-3T3 histone prep. Lane 3: C. elegans embryo cell lysate. Load: 30 µg per lane. Primary antibody: Histone H3 [Trimethyl Lys27, p Ser28] at 1 µg/ml for overnight at 4°C. Secondary antibody: IRDye800™ rabbit secondary antibody at 1:10,000 for 45 min at RT. Block: 5% BLOTTO overnight at 4°C. Predicted/Observed size: ~15 kDa. Other band(s): None.

References

- Mika A et al. Epigenetic changes at the Birc5 promoter induced by YM155 in synovial sarcoma. *J Clin Med.* (2019)

Disclaimer

This product is for research use only and is not intended for therapeutic or diagnostic applications. Please contact a technical service representative for more information. All products of animal origin manufactured by Rockland Immunochemicals are derived from starting materials of North American origin. Collection was performed in United States Department of Agriculture (USDA) inspected facilities and all materials have been inspected and certified to be free of disease and suitable for exportation. All properties listed are typical characteristics and are not specifications. All suggestions and data are offered in good faith but without guarantee as conditions and methods of use of our products are beyond our control. All claims must be made within 30 days following the date of delivery. The prospective user must determine the suitability of our materials before adopting them on a commercial scale. Suggested uses of our products are not recommendations to use our products in violation of any patent or as a license under any patent of Rockland Immunochemicals, Inc. If you require a commercial license to use this material and do not have one, then return this material, unopened to: Rockland Inc., P.O. BOX 5199, Limerick, Pennsylvania, USA.