

Datasheet for 600-401-I55**Histone H3 K4me2/phospho T3 Antibody****Overview**

Description:	Anti-Histone H3 [Dimethyl Lys4, p Thr3] (RABBIT) Antibody - 600-401-I55
Item No.:	600-401-I55
Size:	50 µg
Applications:	ChIP, Dot Blot, IF, Multiplex, WB
Reactivity:	Human, Mouse
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, the dimethylation of H3 lysine 4 (H3 K4Me2) and phosphorylation of threonine 3 (H3 pT3) are known marks of transcriptional activation and mitosis, respectively. While H3K4 has many known modifying enzymes (Set1, Set7/9, MLL, ASH1), Haspin is the only known modifier for H3T3. Recent findings also demonstrate that pT3 can promote binding of survivin in the nucleosome. 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 dimethyl Lys4 pT3 antibody, H3.3B, H3.3AH3F3H3F3B, H3 histone, family 3A, histone H3.3, MGC87782, MGC87783, H3K4me2/pT3
Host Species:	Rabbit
Clonality:	Polyclonal
Format:	IgG

Target Details

Gene Name:	HIST2H3C
Reactivity:	Human, Mouse
PTM Specificity:	Dual Modification
Immunogen Type:	Conjugated Peptide
Immunogen:	Histone H3 [Dimethyl Lys4, p Thr3] affinity purified antibody was prepared from whole rabbit serum produced by repeated immunizations with synthetic dimethylated/phosphorylated peptides surrounding Lysine 4 and Threonine 3 of human Histone H3.2.
Purity/Specificity:	Anti-Histone H3 [Dimethyl Lys4, p Thr3] was affinity purified from monospecific antiserum by immunoaffinity chromatography. This antibody reacts with human Histone H3.2. 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 [Dimethyl Lys4, p Thr3] antibody is tested for Western Blot, Immunocytochemistry/Immunofluorescence, Chromatin Immunoprecipitation, and Dot Blot. 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:	1:50
IHC:	1:50
WB:	1:500

Formulation

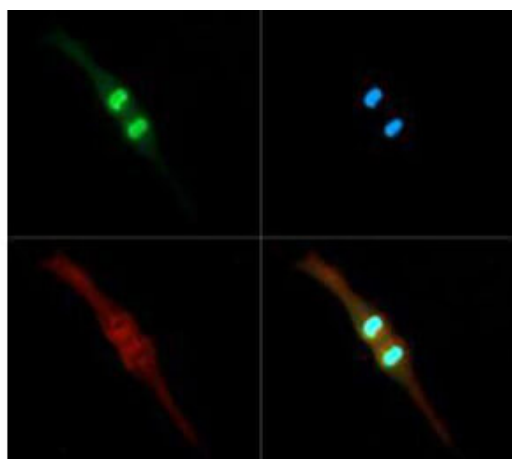
Physical State:	Liquid (sterile filtered)
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Concentration:	0.83 mg/ml by UV absorbance at 280 nm
Buffer:	0.02 M Potassium Phosphate, 0.15 M Sodium Chloride, pH 7.2
Preservative:	0.01% (w/v) Sodium Azide
Stabilizer:	30% Glycerol

Shipping & Handling

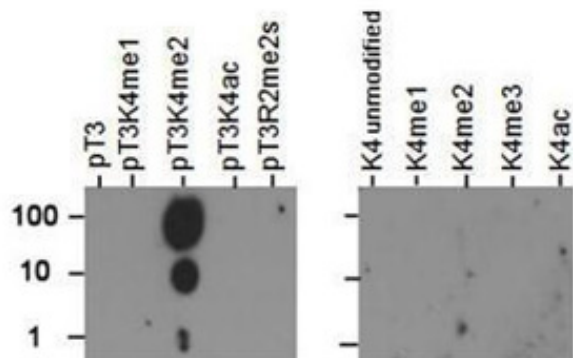
Shipping Condition:	Dry Ice
Storage Condition:	Store vial at -20° C prior to opening. Aliquot contents and freeze at -20° C or below for extended storage. Avoid cycles of freezing and thawing. Centrifuge product if not completely clear after standing at room temperature. This product is stable for several 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



Immunofluorescence Microscopy

Immunofluorescence Microscopy of Rabbit Anti-Histone H3 [p Thr3, Dimethyl Lys4] Antibody. Tissue: HeLa cells.
 Fixation: 0.5% PFA. Antigen retrieval: Not required. Primary antibody: Histone H3 [p Thr3, Dimethyl Lys4] antibody at a 1:50 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 Thr3, Monomethyl Lys4] is nuclear and chromosomal. Staining: Histone H3 [p Thr3, Dimethyl Lys4] is expressed in green while the nuclei and alpha-tubulin were coexpressed with DAPI (blue) and Dylight 550 (red).

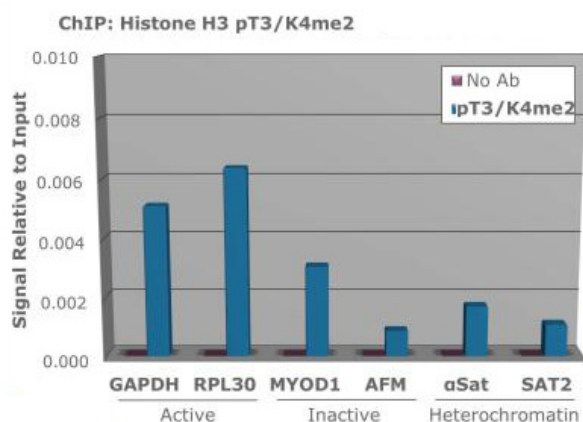


Dot Blot

Dot Blot of Rabbit Histone H3 [p Thr3, Dimethyl Lys4]

Antibody. Antigen: Methylated forms of the immunizing peptide. Load: 1, 10, and 100 picomoles of peptide.

Primary antibody: Histone H3 [p Thr3, Dimethyl Lys4] antibody at 1:1000 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.



ChIP

Chromatin Immunoprecipitation of Rabbit Anti-Histone H3 [p Thr3, Dimethyl Lys4] Antibody. Chromatin from one million formaldehyde cross-linked Hela cells was used with 2ug of Anti-Histone H3 K4 Me2 pT3 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.



Western Blot

Western Blot of Rabbit Anti-Histone H3 [p Thr3, Dimethyl Lys4] Antibody. Lane 1: NIH-3T3 histone prep lysate. Load: 30 µg per lane. Primary antibody: Histone H3 [p Thr3, Dimethyl Lys4] at 1:50 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.

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.