Product Datasheet

Histone H3 Antibody (1B1-B2) - BSA Free
NBP2-36468

Unit Size: 0.1 mg

Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.

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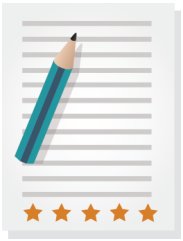
Updated 10/23/2024 v.20.1



NBP2-36468

Histone H3 Antibody (1B1-B2) - BSA Free

Product Information

Unit Size	0.1 mg
Concentration	1.0 mg/ml
Storage	Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.
Clonality	Monoclonal
Clone	1B1-B2
Preservative	0.02% Sodium Azide
Isotype	IgG3 Kappa
Purity	Protein G purified
Buffer	PBS
Target Molecular Weight	15 kDa

Product Description

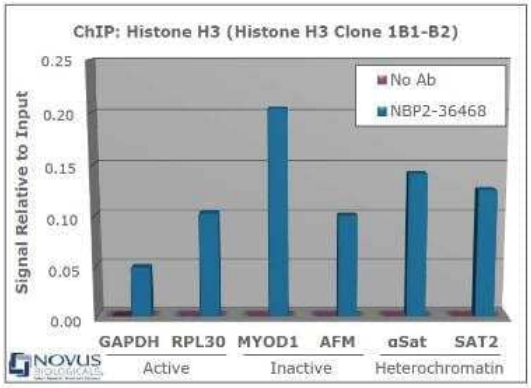
Host	Mouse
Gene ID	126961
Gene Symbol	H3C14
Species	Human, Mouse, Rat
Immunogen	This Histone H3 antibody (1B1-B2) was raised against synthetic peptide corresponding to the C-terminus of human Histone H3.

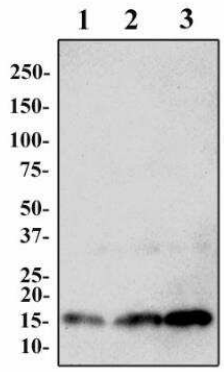
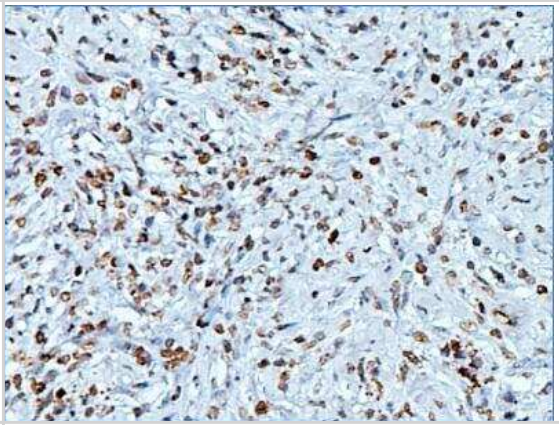
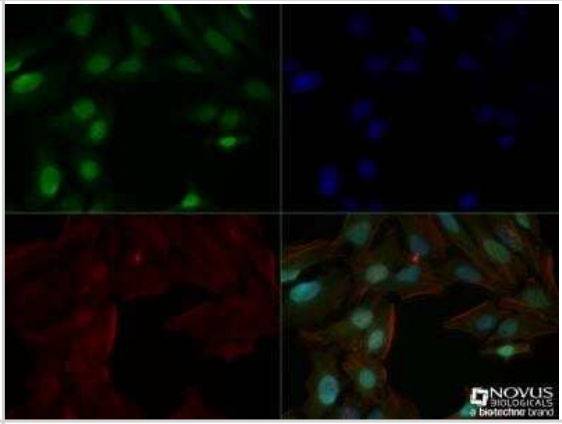
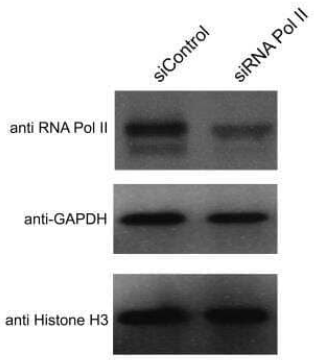
Product Application Details

Applications	Western Blot, Immunocytochemistry/ Immunofluorescence, Immunohistochemistry, Immunohistochemistry-Paraffin, Chromatin Immunoprecipitation (ChIP), Single Cell Western
Recommended Dilutions	Western Blot 1:1000, Immunohistochemistry 1:200-1:500, Immunocytochemistry/ Immunofluorescence 1:200 -1:500, Immunohistochemistry-Paraffin 1:200-1:500, Chromatin Immunoprecipitation (ChIP), Single Cell Western 100 ug/ml

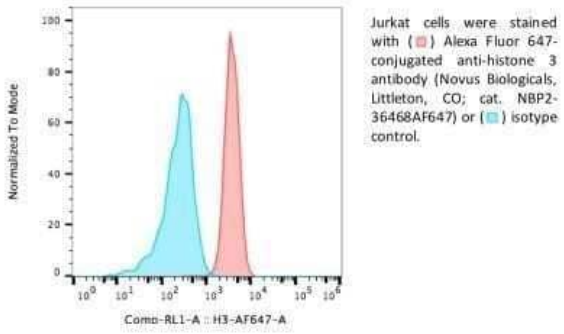
Images

Chromatin Immunoprecipitation: Histone H3 Antibody (1B1-B2) [NBP2-36468] - Chromatin from one million formaldehyde cross-linked HeLa cells was precipitated using 2 ug of NBP2-36468 and 25 ul of magnetic IgG beads, using standard ChIP methods. A similar sample containing no antibody was included as a negative control. Immunoprecipitated DNA was quantified using quantitative real-time PCR and SYBR green dye, then normalized to the non-precipitated input chromatin. Representative target genes from active, inactive and heterochromatic regions of the genome show amplification, indicative of the presence of Histone H3.



Western Blot: Histone H3 Antibody (1B1-B2) [NBP2-36468] - Western blot image of Anti-Histone H3 antibody (clone 1B1-B2). Whole cell protein from HeLa (lane 1), NIH-3T3 (lane 2) and PC12 (lane 3) were separated by 4-15% SDS-PAGE and transfered to PVDF membrane. The membrane was probed with purified Anti-Histone H3 antibody at 2 ug/ml and detected with an HRP conjugated anti-mouse secondary using chemiluminescence. Observed molecular weight is ~15 kDa.	
Immunohistochemistry-Paraffin: Histone H3 Antibody (1B1-B2) [NBP2-36468] - Analysis of Histone H3 in human breast cancer using DAB with hematoxylin counterstain.	
Immunocytochemistry/Immunofluorescence: Histone H3 Antibody (1B1-B2) [NBP2-36468] - HeLa cells were fixed for 10 minutes using 10% formalin and then permeabilized for 5 minutes using 1X TBS + 0.5% Triton-X100. The cells were incubated with anti-Histone H3 (1B1-B2) NBP2-36468 at a 1:200 dilution overnight at 4C and detected with an anti-mouse Dylight 488 (Green) at a 1:500 dilution. Actin was detected with Phalloidin 568 (Red) at a 1:200 dilution. Nuclei were counterstained with DAPI (Blue). Cells were imaged using a 40X objective.	
Western Blot: Histone H3 Antibody (1B1-B2) [NBP2-36468] - Analysis in Hela cells using siRNA to knockdown Pol II, controls are GAPDH and Histone H3. Theoretical molecular weight of Histone H3 is 15 kDa. Image from a verified customer review.	

Flow Cytometry: Histone H3 Antibody (1B1-B2) - BSA Free [NBP2-36468] - Analysis using Alexa Fluor (R) 647 conjugate of NBP2-36468. Jurkat cells stained with Alexa Fluor 647 conjugated Histone H3 antibody (red) or isotype control (blue). Flow cytometry image submitted by a verified customer review.



Publications

Laspata N, Kaur P, Mersaoui SY et al. PARP1 associates with R-loops to promote their resolution and genome stability Nucleic acids research 2023-02-16 [PMID: 36794853] (WB, Human)

Sotty J, Garcon G, Denayer F et al. Toxicological effects of ambient fine (PM2.5-0.18) and ultrafine (PM0.18) particles in healthy and diseased 3D organo-typic mucocilary-phenotype models Environ Res 2019-07-26 [PMID: 31344532]

Procedures

Western Blot Protocol for Histone H3 Antibody (NBP2-36468)
Western Blot Protocol

1. Perform SDS-PAGE on samples to be analyzed, loading 10-25 ug of total protein per lane.
2. Transfer proteins to PVDF membrane according to the instructions provided by the manufacturer of the membrane and transfer apparatus.
3. Stain the membrane with Ponceau S (or similar product) to assess transfer success, and mark molecular weight standards where appropriate.
4. Rinse the blot TBS -0.05% Tween 20 (TBST).
5. Block the membrane in 5% Non-fat milk in TBST (blocking buffer) for at least 1 hour.
6. Wash the membrane in TBST three times for 10 minutes each.
7. Dilute primary antibody in blocking buffer and incubate overnight at 4C with gentle rocking.
8. Wash the membrane in TBST three times for 10 minutes each.
9. Incubate the membrane in diluted HRP conjugated secondary antibody in blocking buffer (as per manufacturer's instructions) for 1 hour at room temperature.
10. Wash the blot in TBST three times for 10 minutes each (this step can be repeated as required to reduce background).
11. Apply the detection reagent of choice in accordance with the manufacturers instructions.

Immunocytochemistry/Immunofluorescence Protocol for Histone H3 Antibody (NBP2-36468)
Immunocytochemistry Protocol

Culture cells to appropriate density in 35 mm culture dishes or 6-well plates.

1. Remove culture medium and wash the cells briefly in PBS. Add 10% formalin to the dish and fix at room temperature for 10 minutes.
2. Remove the formalin and wash the cells in PBS.
3. Permeablize the cells with 0.1% Triton X100 or other suitable detergent for 10 min.
4. Remove the permeablization buffer and wash three times for 10 minutes each in PBS. Be sure to not let the specimen dry out.
5. To block nonspecific antibody binding, incubate in 10% normal goat serum from 1 hour to overnight at room temperature.
6. Add primary antibody at appropriate dilution and incubate overnight at 4C.
7. Remove primary antibody and replace with PBS. Wash three times for 10 minutes each.
8. Add secondary antibody at appropriate dilution. Incubate for 1 hour at room temperature.
9. Remove secondary antibody and replace with PBS. Wash three times for 10 minutes each.
10. Counter stain DNA with DAPi if required.

Immunohistochemistry-Paraffin Protocol for Histone H3 Antibody (NBP2-36468)
Immunohistochemistry-Paraffin Embedded Sections

Antigen Unmasking:
Bring slides to a boil in 10 mM sodium citrate buffer (pH 6.0) then maintain at a sub-boiling temperature for 10 minutes. Cool slides on bench-top for 30 minutes (keep slides in the sodium citrate buffer all the time).

Staining:
1. Wash sections in deionized water three times for 5 minutes each.
2. Wash sections in PBS for 5 minutes.
3. Block each section with 100-400 ul blocking solution (1% BSA in PBS) for 1 hour at room temperature.
4. Remove blocking solution and add 100-400 ul diluted primary antibody. Incubate overnight at 4 C.
5. Remove antibody solution and wash sections in wash buffer three times for 5 minutes each.
6. Add 100-400 ul HRP polymer conjugated secondary antibody. Incubate 30 minutes at room temperature.
7. Wash sections three times in wash buffer for 5 minutes each.
8. Add 100-400 ul DAB substrate to each section and monitor staining closely.
9. As soon as the sections develop, immerse slides in deionized water.
10. Counterstain sections in hematoxylin.
11. Wash sections in deionized water two times for 5 minutes each.
12. Dehydrate sections.
13. Mount coverslips.

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Products Related to NBP2-36468

NB800-PC1	HeLa Whole Cell Lysate
HAF007	Goat anti-Mouse IgG Secondary Antibody [HRP]
NB720-B	Rabbit anti-Mouse IgG (H+L) Secondary Antibody [Biotin]
NBP1-96978	Mouse IgG3 Kappa Light Chain Isotype Control (MG3K)

Limitations
This product is for research use only and is not approved for use in humans or in clinical diagnosis. Primary Antibodies are guaranteed for 1 year from date of receipt.

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