

Product Datasheet

Histone H3 Antibody NB21-1012SS

Unit Size: 0.025 mg

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

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NB21-1012SS

Histone H3 [p Thr3, Monomethyl Lys4] Antibody

Product Information	
Unit Size	0.025 mg
Concentration	0.98 mg/ml
Storage	Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.
Clonality	Polyclonal
Preservative	0.05% Sodium Azide
Purity	Immunogen affinity purified
Buffer	PBS, 30% glycerol

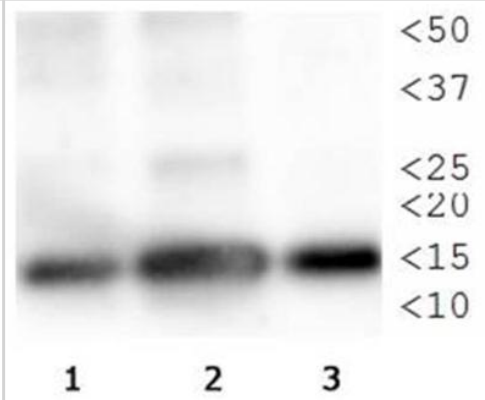
Product Description	
Host	Rabbit
Gene ID	126961
Gene Symbol	HIST2H3C
Species	Human, Mouse, C. elegans
Species Reactivity	Human, mouse, and C. elegans. Predicted to react with many species including rat, chicken, Xenopus, Drosophila, and plant based on 100% sequence homology.
Marker	Nuclear Marker
Immunogen	Synthetic monomethylated/phosphorylated peptides surrounding Lysine 4 and Threonine 3 of human Histone H3.2 [Swiss Prot Q71DI3].
Notes	0.05 mg of HeLa histone preps will be included in the shipment along with the primary antibody as an appropriate positive control. For SDS-PAGE with the positive control we recommend loading 0.01 mg per lane. Epi-Plus antibody production in collaboration with Rockland Immunochemicals Inc.

Product Application Details	
Applications	Western Blot, Dot Blot, Immunocytochemistry/Immunofluorescence, Chromatin Immunoprecipitation (Negative)
Recommended Dilutions	Chromatin Immunoprecipitation (Negative), Dot Blot 1:1000, Immunocytochemistry/Immunofluorescence 1:100, Western Blot 1:500
Application Notes	This Histone H3 H3 K4me1/pT3 antibody is useful for Dot Blot, Western Blot, and Immunocytochemistry/Immunofluorescence. In Western Blot, a band is seen ~15kDa in HeLa histone prep, NIH 3T3 histone prep, and C. elegans embryo lysate. In ICC/IF, nuclear staining is observed in HeLa cells. This antibody is not recommended for Chromatin Immunoprecipitation.

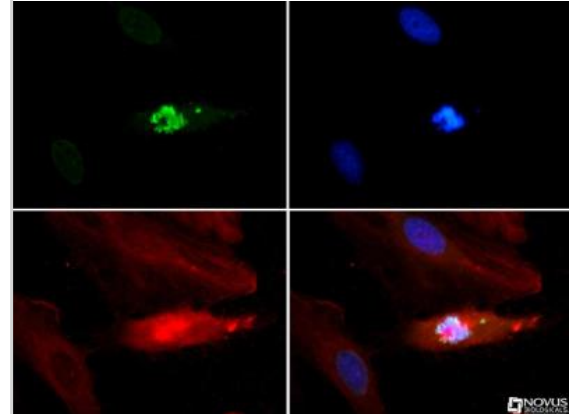


Images

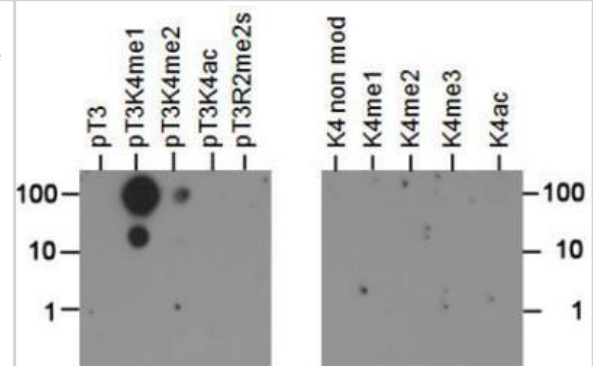
Western Blot: Histone H3 [p Thr3, Monomethyl Lys4] Antibody [NB21-1012] - WB analysis of Histone H3 pT3/K4me1 in: 1. HeLa histone prep, 2. NIH 3T3 histone prep and 3. C. elegans embryo lysate.



Immunocytochemistry/Immunofluorescence: Histone H3 [p Thr3, Monomethyl Lys4] Antibody [NB21-1012] - Histone H3 pT3/K4me1 antibody was tested in HeLa cells with Dylight 488 (green). Nuclei and alpha-tubulin were counterstained with DAPI (blue) and Dylight 550 (red).



Dot Blot: Histone H3 [p Thr3, Monomethyl Lys4] Antibody [NB21-1012] - Dot Blot analysis of H3pT3/K4me1 using the peptides listed above in the 100, 10 and 1 picomoles of peptide.



Epi-Plus[®]
Multi-Assay Validated

Procedures

Western Blot Protocol specific for H3 R2me1 antibody (NB21-1012)

Western Blot Protocol

1. Perform SDS-PAGE on samples to be analyzed, loading 10 ug of histone preps per lane.
2. Transfer proteins to membrane according to the instructions provided by the manufacturer of the membrane and transfer apparatus.
3. Stain according to standard Ponceau S procedure (or similar product) to assess transfer success, and mark molecular weight standards where appropriate.
4. Rinse the blot.
5. Block the membrane using standard blocking buffer for at least 1 hour.
6. Wash the membrane in wash buffer three times for 10 minutes each.
7. Dilute primary antibody in blocking buffer and incubate 1 hour at room temperature.
8. Wash the membrane in wash buffer three times for 10 minutes each.
9. Apply the diluted HRP conjugated secondary antibody in blocking buffer (as per manufacturers instructions) and incubate 1 hour at room temperature.
10. Wash the blot in wash buffer 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.

Note: Tween-20 can be added to the blocking or antibody dilution buffer at a final concentration of 0.05-0.2%.

*The above information is only intended as a guide. The researcher should determine what protocol best meets their needs. Please follow safe laboratory procedures.

Immunocytochemistry/Immunofluorescence Protocol for Histone H3 Antibody (NB21-1012)

Immunocytochemistry Protocol

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

1. Remove culture medium and add 10% formalin to the dish. Fix at room temperature for 30 minutes.
2. Remove the formalin and add ice cold methanol. Incubate for 5-10 minutes.
3. Remove methanol and add washing solution (i.e. PBS). Be sure to not let the specimen dry out. Wash three times for 10 minutes.
4. To block nonspecific antibody binding incubate in 10% normal goat serum from 1 hour to overnight at room temperature.
5. Add primary antibody at appropriate dilution and incubate at room temperature from 2 hours to overnight at room temperature.
6. Remove primary antibody and replace with washing solution. Wash three times for 10 minutes.
7. Add secondary antibody at appropriate dilution. Incubate for 1 hour at room temperature.
8. Remove antibody and replace with wash solution, then wash for 10 minutes. Add Hoechst 33258 to wash solution at 1:25,000 and incubate for 10 minutes. Wash a third time for 10 minutes.
9. Cells can be viewed directly after washing. The plates can also be stored in PBS containing Azide covered in Parafilm (TM). Cells can also be cover-slipped using Fluoromount, with appropriate sealing.

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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.

For more information on our guarantee, please visit www.novusbio.com/guarantee.

