

# Product Datasheet

## Histone H3 [p Thr3, Monomethyl Lys4] Antibody - BSA Free NB21-1012

Unit Size: 0.05 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

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**NB21-1012**

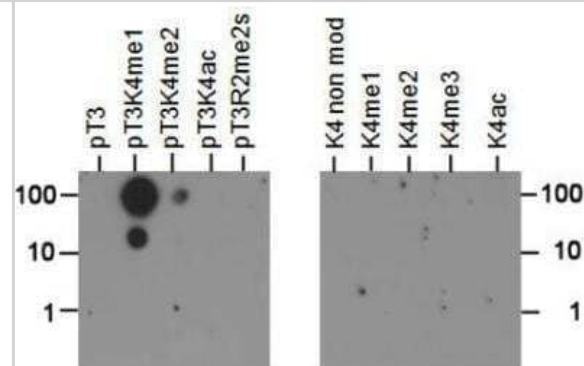
Histone H3 [p Thr3, Monomethyl Lys4] Antibody - BSA Free

| <b>Product Information</b>         |  |
|------------------------------------|--|
| <b>Unit Size</b>                   | 0.05 mg  |
| <b>Concentration</b>               | 0.98 mg/ml   |
| <b>Storage</b>                     | Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.   |
| <b>Clonality</b>                   | Polyclonal   |
| <b>Preservative</b>                | 0.05% Sodium Azide   |
| <b>Isotype</b>                     | IgG  |
| <b>Purity</b>                      | Immunogen affinity purified  |
| <b>Buffer</b>                      | PBS, 30% Glycerol  |
| <b>Target Molecular Weight</b>     | 15 kDa   |
| <b>Product Description</b>         |  |
| <b>Host</b>                        | Rabbit   |
| <b>Gene ID</b>                     | 126961   |
| <b>Gene Symbol</b>                 | H3C14  |
| <b>Species</b>                     | Human, Mouse, C. elegans   |
| <b>Immunogen</b>                   | This Histone H3 [p Thr3, Monomethyl Lys4] antibody was raised against synthetic monomethylated/phosphorylated peptides surrounding Lysine 4 and Threonine 3 of human Histone H3.2 [Swiss Prot Q71DI3]. |
| <b>Notes</b>                       | Epi-Plus antibody production in collaboration with Rockland Immunochemicals Inc.   |
| <b>Product Application Details</b> |  |
| <b>Applications</b>                | Western Blot, Dot Blot, Immunocytochemistry/ Immunofluorescence, Chromatin Immunoprecipitation (Negative)  |
| <b>Recommended Dilutions</b>       | Western Blot 1:500, Immunocytochemistry/ Immunofluorescence 1:100, Dot Blot 1:1000, Chromatin Immunoprecipitation (Negative)   |
| <b>Application Notes</b>           | In Western Blot, a band is seen at ~15kDa in HeLa histone prep, NIH 3T3 histone prep, and C. elegans embryo lysate. In ICC/IF, nuclear staining is observed in HeLa cells.                             |

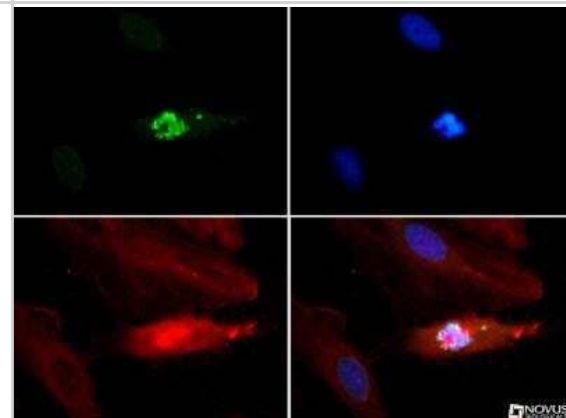


## Images

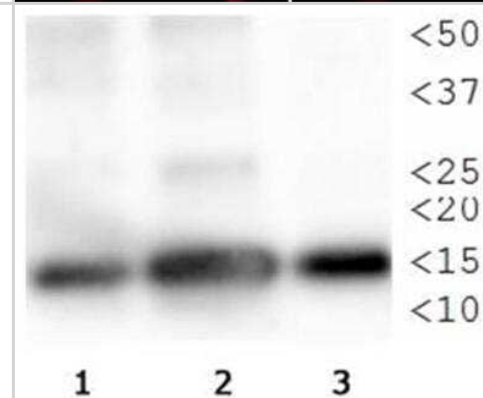
Dot Blot: Histone H3 [p Thr3, Monomethyl Lys4] Antibody [NB21-1012] - Analysis of H3pT3/K4me1 using the peptides listed above in the 100, 10 and 1 picomoles of peptide.



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



Western Blot: Histone H3 [p Thr3, Monomethyl Lys4] Antibody [NB21-1012] - Analysis of Histone H3 pT3/K4me1 in: 1: HeLa histone prep. 2: NIH 3T3 histone prep. 3: C. elegans embryo lysate. Observed molecular weight is ~15 kDa.



## Procedures

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

Histone H3 [p Thr3, Monomethyl Lys4] Antibody:

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)

Histone H3 [p Thr3, Monomethyl Lys4] Antibody:

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.

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



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### **Products Related to NB21-1012**

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|            |  |
|------------|--|
| HAF008     | Goat anti-Rabbit IgG Secondary Antibody [HRP]          |
| NB7160     | Goat anti-Rabbit IgG (H+L) Secondary Antibody [HRP]    |
| NBP2-24891 | Rabbit IgG Isotype Control                             |
| NB21-1012B | Histone H3 [p Thr3, Monomethyl Lys4] Antibody [Biotin] |

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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.

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