

# Product Datasheet

## Histone H3 [p Thr6, Trimethyl Lys9] Antibody - BSA Free NB21-1054

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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**NB21-1054**

Histone H3 [p Thr6, Trimethyl Lys9] Antibody - BSA Free

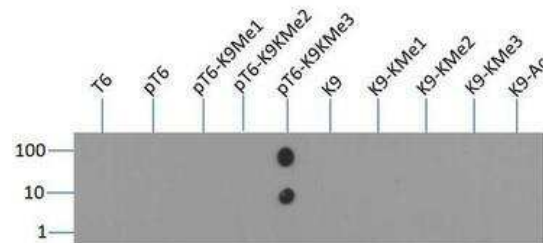
Product Information	
Unit Size	0.05 mg
Concentration	0.55 mg/ml
Storage	Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.
Clonality	Polyclonal
Preservative	0.05% Sodium Azide
Isotype	IgG
Purity	Immunogen affinity purified
Buffer	PBS and 30% Glycerol
Target Molecular Weight	15 kDa

Product Description	
Host	Rabbit
Gene ID	126961
Gene Symbol	H3C14
Species	Human, Mouse, C. elegans
Immunogen	This Histone H3 [p Thr6, Trimethyl Lys9] antibody was raised against synthetic trimethylated/phosphorylated peptide surrounding Lysine 9 / Threonine 6 of human Histone H3.2 [Swiss Prot Q71DI3].
Notes	Epi-Plus antibody production in collaboration with Rockland Immunochemicals Inc.

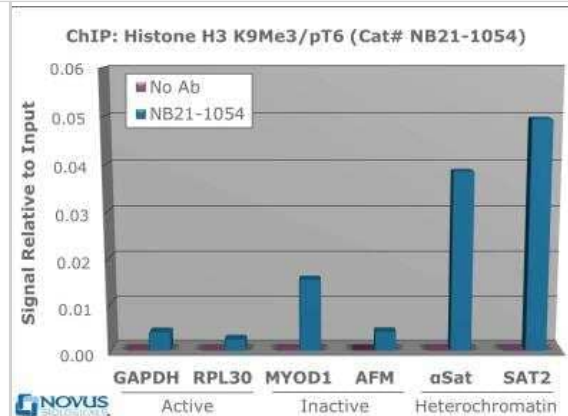
Product Application Details	
Applications	Western Blot, Dot Blot, Immunocytochemistry/ Immunofluorescence, Chromatin Immunoprecipitation (ChIP)
Recommended Dilutions	Western Blot 1:500, Immunocytochemistry/ Immunofluorescence 1:200, Dot Blot 1:1000, Chromatin Immunoprecipitation (ChIP) 2-5 ug per million cells
Application Notes	In Western Blot, a band is seen at ~15kDa in HeLa histone prep and C. elegans embryo lysate. The observed molecular weight of the protein may vary from the listed predicted molecular weight due to post translational modifications, post translation cleavages, relative charges, and other experimental factors.

**Images**

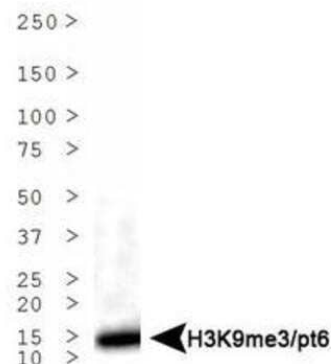
Dot Blot: Histone H3 [p Thr6, Trimethyl Lys9] Antibody [NB21-1054] - Analysis of H3K9me3/pT6 in picomoles of peptide.



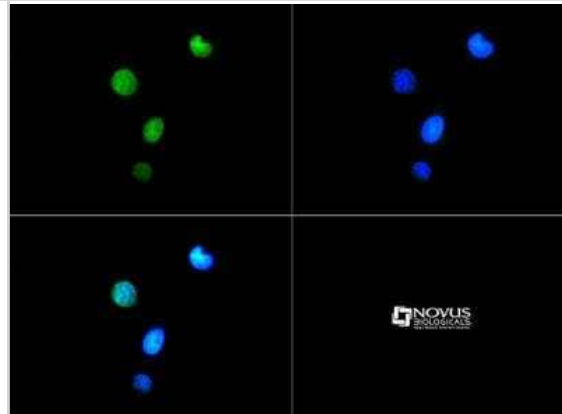
**Chromatin Immunoprecipitation: Histone H3 [p Thr6, Trimethyl Lys9] Antibody [NB21-1054]** - Chromatin from one million formaldehyde cross-linked HeLa cells was used with 2ug of NB21-1054 and 20ul of magnetic IgG beads per immunoprecipitation. A no antibody (No Ab) control was also used. Immunoprecipitated 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.



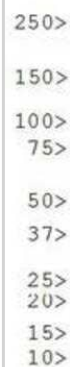
**Western Blot: Histone H3 [p Thr6, Trimethyl Lys9] Antibody [NB21-1054]** - Analysis of H3K9me3/pT6 in HeLa histone preps. Observed molecular weight is ~15 kDa.



**Immunocytochemistry/Immunofluorescence: Histone H3 [p Thr6, Trimethyl Lys9] Antibody [NB21-1054]** - Histone H3.2 K9me3 pT6 antibody was tested at 1:50 in HeLa cells with FITC (green). Nuclei were counterstained with DAPI (blue).



**Western Blot: Histone H3 [p Thr6, Trimethyl Lys9] Antibody [NB21-1054]** - Analysis of H3K9Me3/pT6 in *C. elegans* embryo lysate. Observed molecular weight is ~15 kDa.



## Procedures

### Protocol specific for trimethyl K9/phospho T6 antibody (NB21-1054)

Histone H3 [p Thr6, Trimethyl Lys9] 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%.

### 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.

### Chromatin Immunoprecipitation Protocol

#### Cell Fixation and Preparation

1. Begin with a cell culture that has reached 80% confluency.
2. Add formaldehyde to a final concentration of 1% in growth media and incubate for 10 minutes at room temperature.
3. Add glycine to reach a final concentration of 125 mM in the media. Incubate for 5 minutes at room temperature.
4. Remove all media and wash twice with 20 mL of ice cold PBS.
5. Add 2 mL of ice cold PBS with protease inhibitors\*. Scrape cells into microcentrifuge tube.
6. Spin cells at 4 C for 5 minutes at 800 x g.
7. Remove supernatant and resuspend cells in 750 ul of RIPA lysis buffer containing protease inhibitors\* per 10,000,000 cells (enough for 10 IPs). Incubate at 4 C for 15 minutes.
8. Spin cells at 4 C for 5 minutes at 800 x g.

#### DNA Shearing by Sonication



1. Sonicate crosslinked DNA to fragments sizes of 200-1000 base pairs. Keep samples ice cold to prevent denaturing of chromatin. Conditions for fragmenting must be empirically derived, and vary depending on equipment, cell type, cell density, and cross-linking efficiency.
  2. Centrifuge samples to remove debris at 4 C for 10 minutes at 12,500 x g. Remove supernatant and transfer to a new tube. Discard pellet. Set aside 75 ul of sample for input fraction, which will not go through the subsequent IP steps. The remaining sample can be moved into 75 ul aliquots, each of which is sufficient for a single IP. Although it is preferable to proceed directly to the following steps, sheared chromatin can now be frozen at -80 C for up to 1 month.
- a. Optional: Test the efficiency of the shearing by running 5-10 ul of sample on a 2% agarose gel after reversal of crosslinking, RNase treatment (0.5 mg/ml) and proteinase K treatment (0.1 ug/ul) as described below.

### Chromatin Immunoprecipitation

Recommended controls include: No antibody negative control OR normal IgG negative control, positive control antibody.

1. Dilute each IP sample 1:10 by adding 75 ul sheared chromatin to 675 ul dilution buffer, along with appropriate protease inhibitors\*. Save undiluted input fraction for step 10.
  2. Add 25 ul of fully suspended protein A/G magnetic bead slurry. Do not allow the beads to dry.
  3. Add antibody of interest to the diluted sample. For best results, incubate tubes with rotation at 4 C overnight. Alternatively, incubate at room temperature for 1-2 hours.
  4. Pellet magnetic beads with a magnetic separator and remove the supernatant.
  5. Add 750 ul cold low salt buffer and wash for 5 minutes with rotation. Pellet beads with separator and discard supernatant.
  6. Add 750 ul cold high salt buffer and wash for 5 minutes with rotation. Pellet beads with separator and discard supernatant.
  7. Add 750 ul cold LiCl buffer and wash for 5 minutes with rotation. Pellet beads with separator and discard supernatant.
  8. Add 750 ul cold TE buffer and wash for 5 minutes with rotation. Pellet beads with separator and discard supernatant.
  9. Elute complex by adding 200 ul elution buffer and agitate at RT for 15 minutes. Pellet beads with separator and discard beads. Keep the supernatant.
  10. Add 8 ul of 5M NaCl and 2 ul of proteinase K (10 ug/ul) to each sample to reverse cross-linking. For the input fraction, add 125 ul of elution buffer along with NaCl and proteinase K. Incubate at 62 C for 4 hours or overnight. Incubate at 95 C for 10 minutes to deactivate proteinase K.
- a. Optional: To decrease time, this step may also be performed at 95 C for 20 minutes without proteinase K treatment.

### DNA Purification and Amplification

1. DNA can now be purified with either a commercially available column kit or by phenol/chloroform extraction.
  2. Perform real-time PCR with 2 ul of purified DNA and primers (catalog numbers NBP1-71650, NBP1-71651, NBP1-71652, NBP1-71653, NBP1-71654 and NBP1-71655) per reaction. Dilute input fraction to 1% before PCR. Normalize all IPs and no antibody control IP to adjusted input fraction.
- a. Ex. Raw input CT=30, adjusted input:  $30 - 6.44 = 23.4$   
 b. Antibody CT=28  
 c. Normalized signal relative to input:  $2^{-(23.4-28)} = 0.04$

### Buffers

Dilution Buffer: 0.01% SDS, 1.1% Triton X-100, 1.2 mM EDTA, 16.7 mM Tris-HCL (pH8.1), 167 mM NaCl

Low Salt Wash Buffer: 0.1% SDS, 1% Triton X-100, 2mM EDTA, 20mM Tris-HCl, pH 8.1, 150mM NaCl.

High Salt Wash Buffer: 0.1% SDS, 1% Triton X-100, 2mM EDTA, 20mM Tris-HCl, pH 8.1, 500mM NaCl.

LiCl Wash Buffer: 250mM LiCl, 1% NP-40, 1% Na-deoxycholate, 1mM EDTA, 10mM Tris, pH 8.1.



TE Buffer: 10mM Tris-HCL pH 8.1, 1 mM EDTA

Elution buffer: 1% SDS, 100mM NaHCO<sub>3</sub>

\* Please note that protease inhibitors have variable half-lives and should be freshly prepared as applicable.

\*\*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 NB21-1054

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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
NB21-1054B	Histone H3 [Trimethyl Lys9, p Thr6] 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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