

Product Datasheet

Histone H4 Antibody NB21-2089SS

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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NB21-2089SS

Histone H4 [Dimethyl Lys20] Antibody

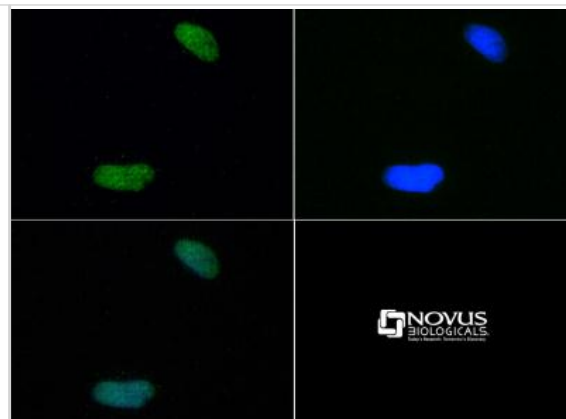
Product Information	
Unit Size	0.025 mg
Concentration	0.53 mg/ml
Storage	Store at 4C short term. Aliquot and store at -20C long term. Avoid freeze-thaw cycles.
Clonality	Polyclonal
Preservative	0.05% Sodium Azide
Purity	Immunogen affinity purified
Buffer	PBS, 30% glycerol

Product Description	
Host	Rabbit
Gene ID	121504
Gene Symbol	HIST4H4
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	H3K20me2
Immunogen	Synthetic dimethylated peptide surrounding Lysine 20 of human Histone H4 [Swiss Prot P62805].
Notes	Epi-Plus antibody production in collaboration with Rockland Immunochemicals Inc.

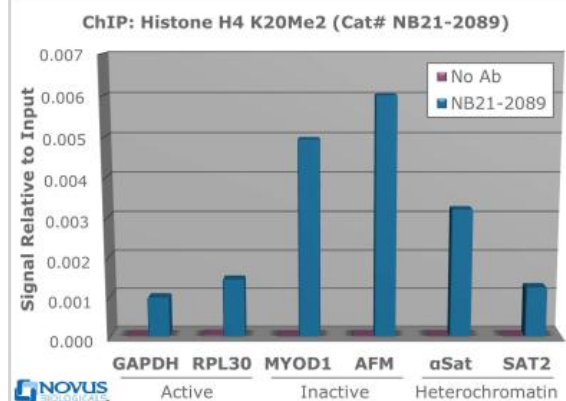
Product Application Details	
Applications	Western Blot, Chromatin Immunoprecipitation, Dot Blot, Immunocytochemistry/Immunofluorescence
Recommended Dilutions	Chromatin Immunoprecipitation 2-5 ug per million cells, Dot Blot 1:1000, Immunocytochemistry/Immunofluorescence 1:50, Western Blot 1:2000
Application Notes	This Histone H4 K20me2 antibody is useful for ChIP, Immunocytochemistry/Immunofluorescence, Dot blot, and Western blot where a band is seen ~13kDa.

Images	
Western Blot: Histone H4 [Dimethyl Lys20] Antibody [NB21-2089] - WB analysis of Histone H4K20me2 in C. elegans embryo lysate.	

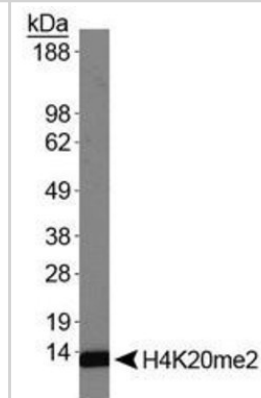
Immunocytochemistry/Immunofluorescence: Histone H4 [Dimethyl Lys20] Antibody [NB21-2089] - Histone H4 K20me2 antibody was tested at 1:50 in HeLa cells with FITC (green). Nuclei (Blue) were counterstained with Dapi (blue).



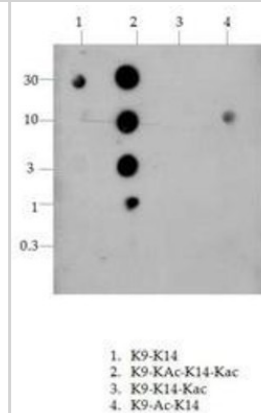
Chromatin Immunoprecipitation: Histone H4 [Dimethyl Lys20] Antibody [NB21-2089] - Chromatin from one million formaldehyde cross-linked HeLa cells was used with 2ug of NB21-2089 and 20ul of magnetic IgG beads per immunoprecipitation. A no antibody (No Ab) control was also used. Immunoprecipitated DNA 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 H4 [Dimethyl Lys20] Antibody [NB21-2089] - WB detection of H4K20me2 in HeLa histone preps.



Dot Blot: Histone H4 [Dimethyl Lys20] Antibody [NB21-2089] - Dot blot analysis of H4K20me2 using peptides stated above in 4, 3, 2 and 1 picomoles of peptide.





Procedures

Western Blot protocol specific for H4K20me2 antibody (NB21-2089)

Western Blot Protocol

1. Perform SDS-PAGE on samples to be analyzed, loading 40 ug of total protein 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 the rabbit anti-H4K20me2 primary antibody (NB21-2089) 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 diluted rabbit-IgG 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/Immunofluorescence Protocol for Histone H4 Antibody (NB21-2089)

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 (NB21-2089)

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 4C for 5 minutes at 800 x g.
7. Remove supernatant and resuspend cells in 750 ul of RIPA lysis buffer containing protease inhibitors* per 1×10^7 cells (enough for 10 IPs). Incubate at 4C for 15 minutes.



8. Spin cells at 4C 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 4C 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 -80C 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 4C 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 NaHCO₃



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





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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.

