

# Product Datasheet

## Histone H3 [Trimethyl Lys27] Antibody - BSA Free NBP2-54614

Unit Size: 50 ug

Store at -20C short term. Aliquot and store at -80C long term. Avoid freeze-thaw cycles.

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**NBP2-54614**

Histone H3 [Trimethyl Lys27] Antibody - BSA Free

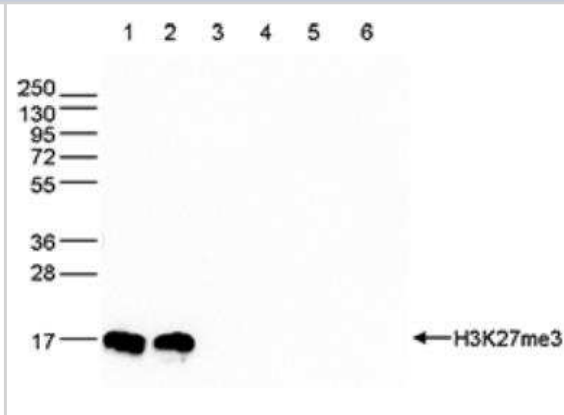
Product Information	
Unit Size	50 ug
Concentration	Please see the vial label for concentration. If unlisted please contact technical services.
Storage	Store at -20C short term. Aliquot and store at -80C long term. Avoid freeze-thaw cycles.
Clonality	Polyclonal
Preservative	0.05% Sodium Azide and 0.05% ProClin 300
Isotype	IgG
Purity	Affinity purified
Buffer	PBS
Target Molecular Weight	15 kDa

Product Description	
Description	Novus Biologicals Rabbit Histone H3 [Trimethyl Lys27] Antibody - BSA Free (NBP2-54614) is a polyclonal antibody validated for use in WB, ELISA, ICC/IF and ChIP. All Novus Biologicals antibodies are covered by our 100% guarantee.
Host	Rabbit
Gene ID	126961
Gene Symbol	H3C14
Species	Human, Mouse, A. thaliana, C. elegans, Drosophila, Plant
Reactivity Notes	Maize, tomato, poplar
Immunogen	This Histone H3 [Trimethyl Lys4] antibody was developed against H3K27me3

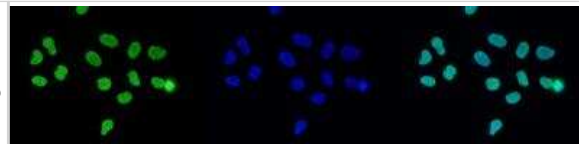
Product Application Details	
Applications	Western Blot, Dot Blot, ELISA, Immunocytochemistry/ Immunofluorescence, Protein Array, Chromatin Immunoprecipitation (ChIP), Chromatin Immunoprecipitation Sequencing
Recommended Dilutions	Western Blot 1:1000, ELISA 1:100 - 1:500, Immunocytochemistry/ Immunofluorescence 1:200, Dot Blot 1:5000, Protein Array 1:5000, Chromatin Immunoprecipitation (ChIP) 1-2 ug/IP per ChIP, Chromatin Immunoprecipitation Sequencing 1 - 2 ug/IP per ChIP

**Images**

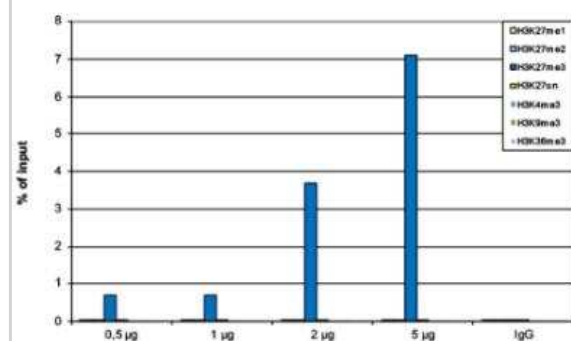
Western Blot: Histone H3 [Trimethyl Lys27] Antibody [NBP2-54614] - Western blot was performed on whole cell (25 ug, lane 1) and histone extracts (15 ug, lane 2) from HeLa cells, and on 1 ug of recombinant histone H2A, H2B, H3 and H4 (lane 3, 4, 5 and 6, respectively) using the antibody against H3K27me3 diluted 1:500 in TBS-Tween containing 5% skimmed milk. Observed molecular weight is ~17 kDa.



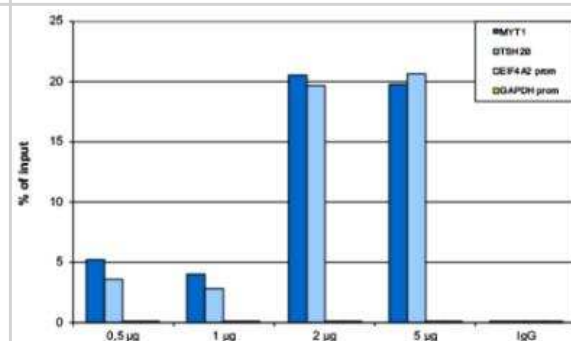
**Immunocytochemistry/Immunofluorescence: Histone H3 [Trimethyl Lys27] Antibody [NBP2-54614]** - Human HeLa cells were stained with the antibody against H3K27me3 and with DAPI. Cells were fixed with 4% formaldehyde for 10' and blocked with PBS/Triton X-100 containing 5% normal goat serum and 1% BSA. The cells were immunofluorescently labelled with the H3K27me3 antibody (left) diluted 1:200 in blocking solution followed by an anti-rabbit antibody conjugated to Alexa Fluor 488. The middle panel shows staining of the nuclei with DAPI. A merge of the two stainings is shown on the right.



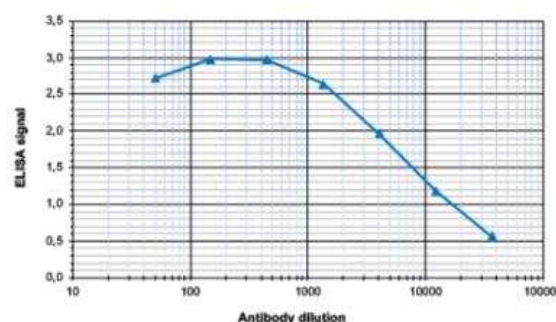
**Chromatin Immunoprecipitation: Histone H3 [Trimethyl Lys27] Antibody [NBP2-54614]** - Recovery of the nucleosomes carrying the H3K27me1, H3K27me2, H3K27me3, H3K4me3, H3K9me3 and H3K36me3 modifications and the unmodified H3K27 as determined by qPCR. The figure clearly shows the antibody is very specific in ChIP for the H3K27me3 modification.



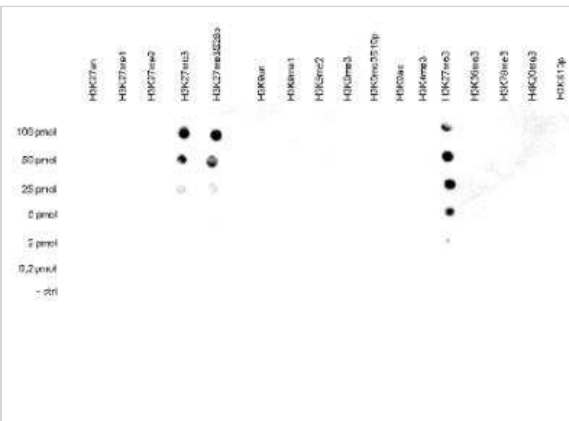
**Chromatin Immunoprecipitation: Histone H3 [Trimethyl Lys27] Antibody [NBP2-54614]** - ChIP assays were performed using human HeLa cells, the antibody against H3K27me3 and optimized PCR primer pairs for qPCR. ChIP was performed with a ChIP-seq kit, using sheared chromatin from 1 million cells. The chromatin was spiked with a panel of in vitro assembled nucleosomes, each containing a specific lysine methylation (SNAP-ChIP K-MetStat Panel, Epicypher). A titration consisting of 0.5, 1, 2 and 5 µg of antibody per ChIP experiment was analyzed. IgG (1 µg/IP) was used as a negative IP control. Quantitative PCR was performed with primers specific for the promoter of the active GAPDH and EIF4A2 genes, used as negative controls, and for the inactive TSH2B and MYT1 genes, used as positive controls. The graph shows the recovery, expressed as a % of input (the relative amount of immunoprecipitated DNA compared to input DNA after qPCR analysis).



**ELISA: Histone H3 [Trimethyl Lys27] Antibody [NBP2-54614]** - To determine the titer of the antibody, an ELISA was performed using a serial dilution of the Diagenode antibody directed against H3K27me3. The antigen used was a peptide containing the histone modification of interest. By plotting the absorbance against the antibody dilution, the titer of the antibody was estimated to be 1:3,000.



Dot Blot: Histone H3 [Trimethyl Lys27] Antibody [NBP2-54614] - A Dot Blot analysis was performed to test the cross reactivity of the antibody against H3K27me3 with peptides containing other modifications of histone H3 and H4 and the unmodified H3K27 sequence. One hundred to 0.2 pmol of the peptide containing the respective histone modification were spotted on a membrane. The antibody was used at a dilution of 1:5,000. The figure shows a high specificity of the antibody for the modification of interest. Please note that the antibody also recognizes the modification if S28 is phosphorylated.





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### **Products Related to NBP2-54614**

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NBP2-33376H	Blue Marker Antibody (6F4-F6) [HRP]
HAF008	Goat anti-Rabbit IgG Secondary Antibody [HRP]
NB7160	Goat anti-Rabbit IgG (H+L) Secondary Antibody [HRP]
NBP2-24891	Rabbit IgG Isotype Control

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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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