

# Product Datasheet

## Histone H3 [Trimethyl Lys27, p Ser28] Antibody NBP2-59161

Unit Size: 100 ul

Store at -20C. Avoid freeze-thaw cycles.

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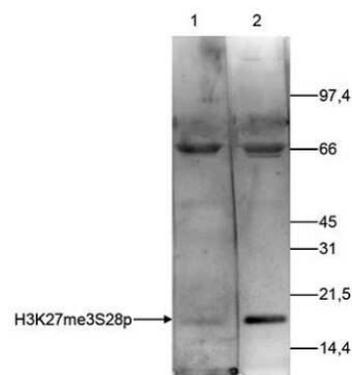


**NBP2-59161****Histone H3 [Trimethyl Lys27, p Ser28] Antibody**

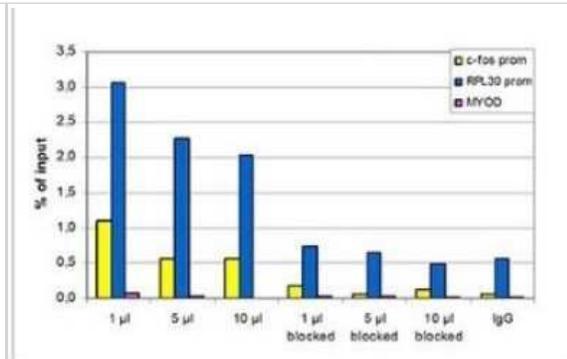
Product Information	
<b>Unit Size</b>	100 ul
<b>Concentration</b>	Please see the vial label for concentration. If unlisted please contact technical services.
<b>Storage</b>	Store at -20C. Avoid freeze-thaw cycles.
<b>Clonality</b>	Polyclonal
<b>Preservative</b>	0.05% Sodium Azide
<b>Isotype</b>	IgG
<b>Purity</b>	Whole Antiserum
<b>Buffer</b>	Serum
<b>Target Molecular Weight</b>	15 kDa
Product Description	
<b>Host</b>	Rabbit
<b>Gene ID</b>	126961
<b>Gene Symbol</b>	H3C14
<b>Species</b>	Human
<b>Immunogen</b>	The exact sequence of the immunogen to this Histone H3 [Trimethyl Lys27, p Ser28] antibody is proprietary.
Product Application Details	
<b>Applications</b>	Western Blot, Chromatin Immunoprecipitation, Dot Blot, ELISA, Immunocytochemistry/ Immunofluorescence, Immunoprecipitation, Immunofluorescence
<b>Recommended Dilutions</b>	Western Blot 1:250 - 1:500, Chromatin Immunoprecipitation 1 uL/IP, ELISA 1:100 - 1:500, Immunocytochemistry/ Immunofluorescence 1:200, Immunoprecipitation 5 ul/IP, Dot Blot 1:20000, Immunofluorescence 1:200

**Images**

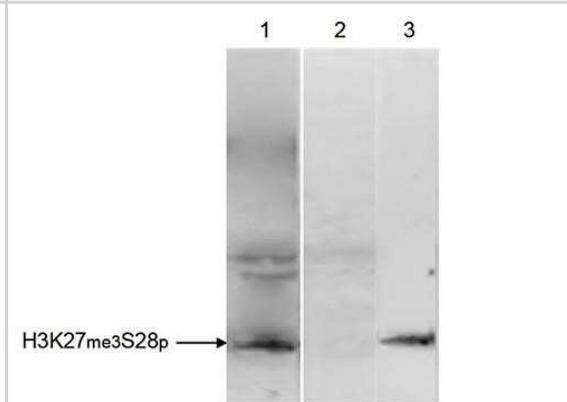
Western Blot: Histone H3 [Trimethyl Lys27, p Ser28] Antibody [NBP2-59161] - Histone extracts of HeLa cells (15 ug) were analysed by Western blot using the antibody against H3K27me3S28p diluted 1:250 in TBS-Tween containing 5% skimmed milk. Lane 2 shows the result of the Western analysis with the antibody; lane 1 shows the same analysis after incubation of the antibody with 750 pmol blocking peptide for 1 hour at room temperature. Observed molecular weight is ~17 kDa.



**Chromatin Immunoprecipitation: Histone H3 [Trimethyl Lys27, p Ser28] Antibody [NBP2-59161]** - ChIP assays were performed using HeLa cells treated with colcemid, the antibody against H3K27me3S28p and optimized PCR primer pairs for qPCR. ChIP was performed using sheared chromatin from 10,000 cells. A titration of the antibody consisting of 1, 5 and 10  $\mu$ L per ChIP experiment was analysed. Additionally, ChIP was performed after incubation of the antibody with 5 nmol blocking peptide for 1 hour at room temperature. IgG (5  $\mu$ g/IP) was used as negative IP control. QPCR was performed with primers for the promoters of the active genes *c-fos* and *RPL30* and for the inactive gene *MYOD*. Figure shows the recovery, expressed as a % of input (the relative amount of immunoprecipitated DNA compared to input DNA after qPCR analysis).



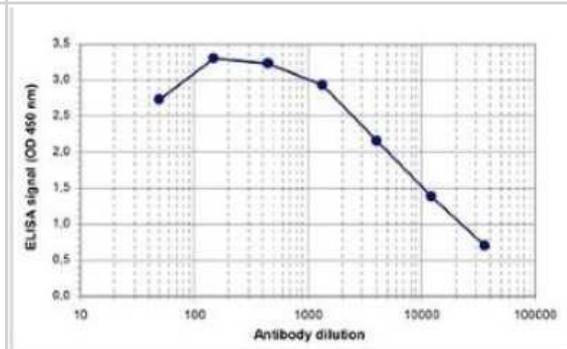
**Western Blot: Histone H3 [Trimethyl Lys27, p Ser28] Antibody [NBP2-59161]** - HeLa cells were treated with colcemid to block the cell cycle in metaphase and were fixed with formaldehyde. Chromatin from 10,000 cells was sheared and used for immunoprecipitation (IP). IP was performed with 5  $\mu$ L of the antibody against H3K27me3S28p. The immunoprecipitated proteins were analysed by Western blot with the antibody diluted 1:500 in TBS-Tween containing 5% skimmed milk. Lane 1 shows the result of the IP; a negative IP control (no antibody added) and a positive control (sheared chromatin from 10,000 cells) are shown in lane 2 and 3, respectively.



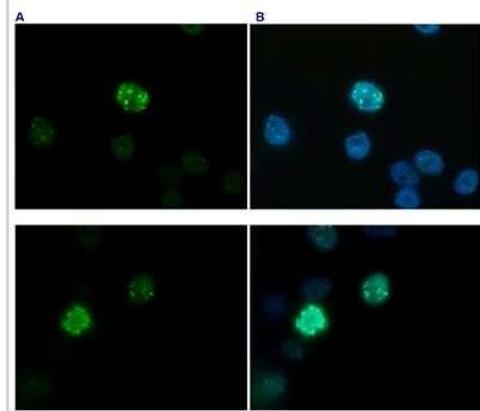
**Dot Blot: Histone H3 [Trimethyl Lys27, p Ser28] Antibody [NBP2-59161]** - A Dot Blot analysis was performed to test the cross reactivity of the antibody against H3K27me3S28p with peptides containing other modifications of histone H3 and H4 and unmodified sequences from histone H3. One hundred to 0.2 pmol of the peptides were spotted on a membrane. The antibody was used at a dilution of 1:20,000. Figure shows a high specificity of the antibody for the peptide containing the modifications of interest.



**ELISA: Histone H3 [Trimethyl Lys27, p Ser28] Antibody [NBP2-59161]** - To determine the titer, an ELISA was performed using a serial dilution of the antibody directed against H3K27me3S28p. The antigen used was a peptide containing the histone modifications of interest. By plotting the absorbance against the antibody dilution, the titer of the antibody was estimated to be 1:8,300.



Immunofluorescence: Histone H3 [Trimethyl Lys27, p Ser28] Antibody [NBP2-59161] - HeLa asynchronous cells were stained with the antibody against H3K27me3S28p and with DAPI. Cells were fixed with formaldehyde, permeabilized with sodium citrate and Triton X-100 and blocked with PBS containing 2.5% BSA. FigureA: cells were immunofluorescently labelled with the H3K27me3S28p antibody (diluted 1:200 and incubated for 1 hour at room temperature) followed by goat anti-rabbit antibody conjugated to DyLight 488. FigureB: the nuclei were stained with DAPI, which specifically labels DNA. Phosphorylation of H3 on serine 28 is increased during late G2 phase and reaches a maximum in metaphase cells. This may explain the different staining intensities of different cells.





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### **Limitations**

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