

# Product Datasheet

## 5-MethylCytosine Antibody (33D3) - BSA Free NBP2-54609

Unit Size: 100 ug

Store at -80C. Avoid freeze-thaw cycles.

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Updated 9/9/2025 v.20.1

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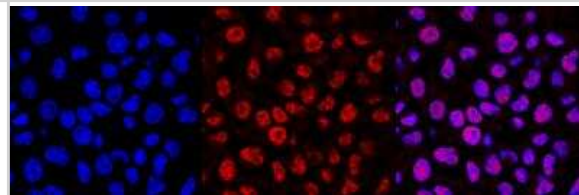


**NBP2-54609****5-MethylCytosine Antibody (33D3) - BSA Free**

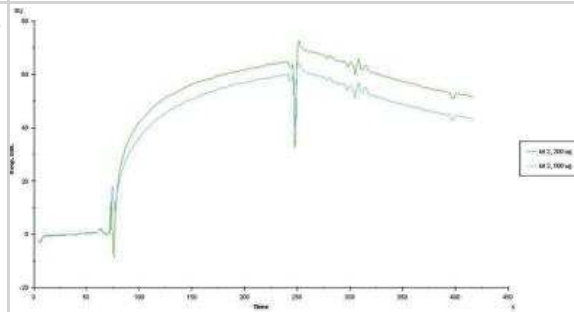
<b>Product Information</b>	
<b>Unit Size</b>	100 ug
<b>Concentration</b>	Please see the vial label for concentration. If unlisted please contact technical services.
<b>Storage</b>	Store at -80C. Avoid freeze-thaw cycles.
<b>Clonality</b>	Monoclonal
<b>Clone</b>	33D3
<b>Preservative</b>	0.05% Sodium Azide
<b>Isotype</b>	IgG1
<b>Purity</b>	Protein A purified
<b>Buffer</b>	PBS
<b>Product Description</b>	
<b>Description</b>	Novus Biologicals Mouse 5-MethylCytosine Antibody (33D3) - BSA Free (NBP2-54609) is a monoclonal antibody validated for use in ICC/IF. All Novus Biologicals antibodies are covered by our 100% guarantee.
<b>Host</b>	Mouse
<b>Species</b>	Human, Mouse, Rat, Bovine
<b>Immunogen</b>	5-methylcytosine
<b>Product Application Details</b>	
<b>Applications</b>	Dot Blot, Immunocytochemistry/ Immunofluorescence, Methylated DNA Immunoprecipitation, Immunofluorescence, Surface Plasmon Resonance
<b>Recommended Dilutions</b>	Immunocytochemistry/ Immunofluorescence 1:500, Dot Blot 1:100, Methylated DNA Immunoprecipitation 0.5 - 1.0 ug/IP, Surface Plasmon Resonance, Immunofluorescence 1:500

## Images

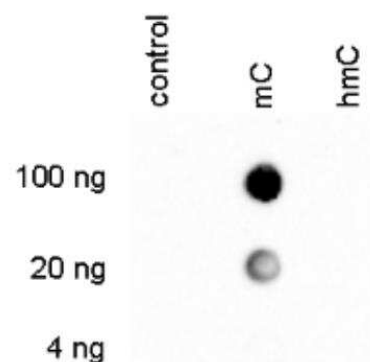
**Immunocytochemistry/Immunofluorescence: 5-MethylCytosine Antibody (33D3) [NBP2-54609]** - HeLa cells were stained with the antibody against 5-mC and with DAPI. Cells were fixed with 4% formaldehyde for 10 minutes and blocked with PBS/TX-100 containing 1% BSA. The cells were immunofluorescently labelled with the 5-mC antibody (middle) diluted 1:500 in blocking solution followed by an anti-mouse antibody conjugated to Alexa Fluor 594. The left panel shows staining of the nuclei with DAPI. A merge of the two stainings is shown on the right.



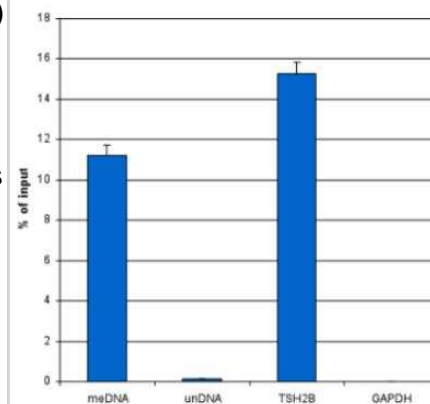
**Surface Plasmon Resonance: 5-MethylCytosine Antibody (33D3) [NBP2-54609]** - A synthesized biotin-labeled 5-mC conjugate was immobilized on a sensorchip. Briefly, two flowcells were prepared by sequential injections of EDC/NHS, streptavidin, and ethanolamine. One of these flowcells served as negative control, while biotinylated 5-mC conjugate was injected in the other one, to get an immobilization level of 55 response units (RU). All SPR experiments were performed, using HBS-N buffer (10 mM HEPES, 150 mM NaCl, pH 7.4), at a flow rate of 5 uL/min. Interaction assays involved injections of 2 different dilutions of the 5-mC monoclonal antibody over the biotinylated 5-mC conjugate and negative control surfaces, followed by a 3 minute washing step with HBS-N buffer. At the end of each cycle, the streptavidin surface was regenerated by injection of 0.1M citric acid (pH 3). The value of the dissociation constant (kd) obtained by global fitting and 1:1 Langmuir model is 65 nM.



**Dot Blot: 5-MethylCytosine Antibody (33D3) [NBP2-54609]** - To demonstrate the specificity of the antibody against 5-mC, a Dot blot analysis was performed using hmC, mC and C controls. 100 to 4 ng (equivalent of 5 to 0.2 pmol of C-bases) of the controls were spotted on a membrane. The antibody was used at a dilution of 1:300. Figure shows a high specificity of the antibody for the methylated control.



**Methylated DNA Immunoprecipitation: 5-MethylCytosine Antibody (33D3) [NBP2-54609]** - Analysis was performed on 1 ug fragmented human genomic DNA using 0.2 ug of the monoclonal antibody against 5-mC. The fragmented DNA was spiked with controls (methylated DNA (meDNA) as a positive and unmethylated DNA (unDNA) as a negative control) prior to performing the IP. QPCR was performed with primer sets specific for the methylated and unmethylated DNA controls, and for a known methylated (TSH2B) and unmethylated (GAPDH) genomic region. The figure shows the recovery expressed as a percent of input (the relative amount of immunoprecipitated DNA compared to input DNA after qPCR analysis).





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### **Products Related to NBP2-54609**

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HAF007	Goat anti-Mouse IgG Secondary Antibody [HRP]
NB7539	Goat anti-Mouse IgG (H+L) Secondary Antibody [HRP]
NBP1-97005-0.5mg	Mouse IgG1 Isotype Control (MG1)
NBP2-62131	5-MethylCytosine ELISA Kit (Colorimetric)

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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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