# antibodies - online.com







# anti-5-Methylcytosine antibody



**Images** 



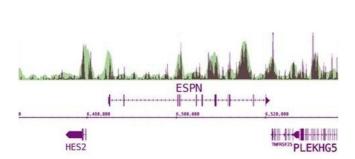
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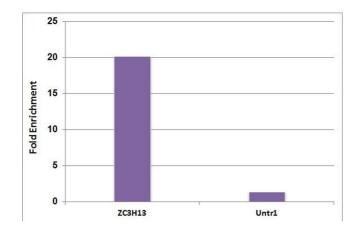
Quantity:	100 μg	
Target:	5-Methylcytosine	
Reactivity:	Human	
Host:	Mouse	
Clonality:	Monoclonal	
Application:	Methylated DNA Immunoprecipitation (MeDIP), Immunofluorescence (IF), Dot Blot (DB)	
Product Details		
Immunogen:	This 5-Methylcytosine (5-mC) antibody was raised against 5-Methyl-cytidine conjugated to KLH and recognizes 5-Methylcytosine.	
Clone:	A1	
Isotype:	IgG1	
Characteristics:	5-Methylcytosine (5-mC) is a modified base that is found in the DNA of plants and vertebrates. DNA methylation is an epigenetic event in which DNA methyltransferases (DNMTs) catalyze the reaction of a methyl group to the fifth carbon of cytosine in a CpG dinucleotide. This modification helps to control gene expression and is also involved in genomic imprinting, while aberrant DNA methylation is often associated with disease. The 5-methylcytidine antibody (Clone A1) has been developed to discriminate between the modified base and its normal cytosine counterpart, allowing for gene promoter methylation analysis. 5-Methylcytosine (5-mC) antibody (mAb) (Clone A1) was raised in a Mouse host. It has been validated for use in Dot blot, Immunofluorescence and Methyl DNA Immunoprecipitation, it has been shown to react with Human samples, but the sequence is not species specific so it should react with a wide	

## **Product Details**

	range of sample types.	
Purification:	Protein A Chromatography	
Target Details		
Target:	5-Methylcytosine	
Alternative Name:	5-Methylcytosine (5-mC)	
Target Type:	Chemical	
Application Details		
Application Notes:	Optimal working dilution should be determined by the investigator.	
Restrictions:	For Research Use only	
Handling		
Buffer:	Purified IgG in PBS with 30 % glycerol and 0.035 % sodium azide.	
Preservative:	Sodium azide	
Precaution of Use:	This product contains Sodium azide: a POISONOUS AND HAZARDOUS SUBSTANCE which	
	should be handled by trained staff only.	
Storage:	-20 °C	
Storage Comment:	Avoid repeated freeze/thaw cycles by aliquoting items into single-use fractions for storage at -	
	20°C for up to 2 years. Keep all reagents on ice when not in storage.	







#### **Dot Blot**

Image 1. 5-Methylcytosine (5-mC) antibody (mAb) tested by dot blot analysis. DNA from the Methylated DNA Standard Kit were spotted (5 ng per spot) on to a positively charged nylon membrane and blotted with 5-Methylcytosine antibody (2 μg/mL dilution). Lane 1: single-stranded unmethylated DNA. Lane 2: single-stranded DNA containing 5-methylcytosine. Lane 3: single-stranded DNA containing 5-hydroxymethylcytosine. Lane 4: double-stranded unmethylated DNA. Lane 5: double-stranded DNA containing 5-methylcytosine. Lane 6: double-stranded DNA 5-hydroxymethylcytosine.

#### **Next-Generation Sequencing**

**Image 2.** Next-Gen sequencing data generated using 5-Methylcytosine (5-mC) antibody (mAb) correlates well with CpG density. DNA was enriched from 1 μg of denatured, adaptor ligated human PC9 cell DNA using 2 μL of the 5-Methylcytosine (5-mC) antibody (mAb). MeDIP DNA was sequenced using the Illumina platform to generate 26 million sequence tags. Tags were mapped to generate a whole-genome DNA methylation profile. The image above shows that the enriched regions (purple peaks) correlate well with CpG density (green overlay).

### **Methylated DNA Immunoprecipitation**

**Image 3.** 5-Methylcytosine (5-mC) antibody (mAb) tested by Methyl-DNA Immunoprecipitation (MeDIP). Methylated DNA derived from the promoter of the APC gene was amplified using 5me-dCTP and PCR. 25pg of methylated DNA was spiked into 500 ng of Msel digested human genomic DNA and subjected to the MeDIP procedure using 5 μg of 5-Methylcytidine antibody or control mouse IgG. Real time quantitative PCR to amplify the methylated APC promoter DNA was performed on the immunoprecipitated DNA and



results plotted as Fold Enrichment over control IgG.