

# DNA Methylation Antibody Panel Pack I

Base Catalog # C20000

## PACK CONTENTS

Component	Size	Shipping Temperature	Storage Upon Receipt	Storage Checklist
<b>5MC33</b> 5-Methylcytosine (5-mC) Monoclonal Antibody [33D3]	25 µg	4°C	-20°C	
<b>5HMC4</b> 5-Hydroxymethylcytosine (5-hmC) Monoclonal Antibody [HMC/4D9]	25 µg	4°C	-20°C	
<b>HGM2</b> HRP-Goat Anti-Mouse Secondary Antibody	50 µg	4°C	-20°C	

## SHIPPING & STORAGE

This product is shipped on frozen ice packs at 4°C. Upon receipt: (1) Store all components at -20°C away from light.

All components of the product are stable for 6 months from the date of shipment, when stored properly.

## 5-Methylcytosine (5-mC) Monoclonal Antibody [33D3] Antibody (Component Cat. #C20000-1-5MC33)

### Background

5-methylcytosine (5-mC) is formed when DNA methyltransferase (DNMT) catalyzes the addition of a methyl group onto the 5-carbon of the cytosine ring, an epigenetic process known as DNA methylation. 5-mC is considered the "fifth" DNA base and this 5-methylcytosine mouse monoclonal antibody is ideal for discriminating between the unmodified cytosine base (C) and the methylated cytosine base (5-mC) for DNA methylation studies. DNA methylation, the major epigenetic modification of eukaryotic genomes, plays an essential role in mammalian development. DNA methylation of promoter regions leads to inactivation of gene function. Also, DNA methylation status varies according to tissue type, and region-specific DNA hypermethylation and global DNA hypomethylation have been demonstrated to play an important role in tumorigenesis.

### Description

Mouse monoclonal antibody to 5-methylcytosine (5-mC), clone 33D3, MeDIP/ChIP-grade, used in DNA methylation studies.

### Concentration

1 mg/ml

### Purification

Protein A

### Immunogen

Ovalbumin-conjugated 5-methylcytosine (5-mC)

### Isotype

IgG1

### Specificity

Modified base 5-methylcytosine (5-mC), a broad range of species.

### Formulation

Purified IgG in 10 mM phosphate buffer, 150 mM NaCl, pH 7.4.

### Storage

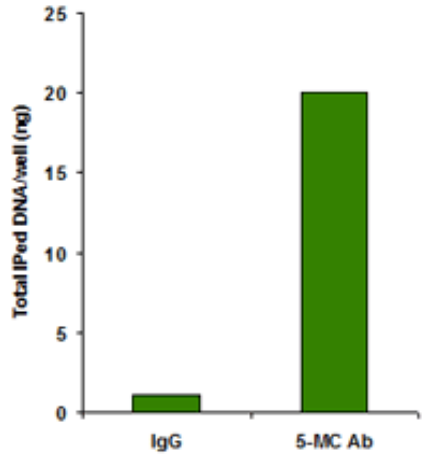
4°C, stable for 6 months from the date of shipment. For long-term storage, aliquot and store at -20°C. Avoid repeated freezing and thawing. Multiple freeze/thaw cycles may result in decreased performance.

### Alternative Names

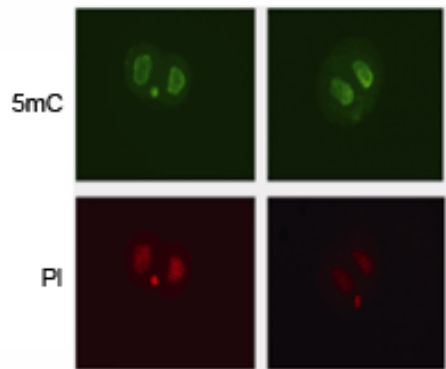
5-methylcytidine, anti-5-methylcytidine, anti-5-methylcytosine, anti-5mC, anti-5-mC, anti-5meC, anti-5-meC, 5mC, 5meC, 5-mC, 5'-methyl-2'-deoxycytidine, 5MedCyd

### Application & Suggested Dilutions\*

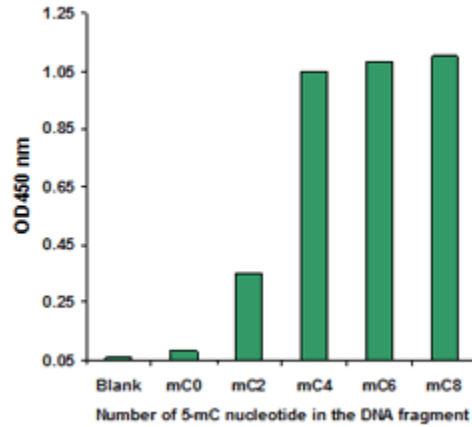
Dot Blot: 1:1000-1:2000; Immunohistochemistry: 1:100-1:500; Immunofluorescence: 1:100-1:500; ELISA: 1:1000-1:2000; MeDIP: 0.5-1 µg/reaction



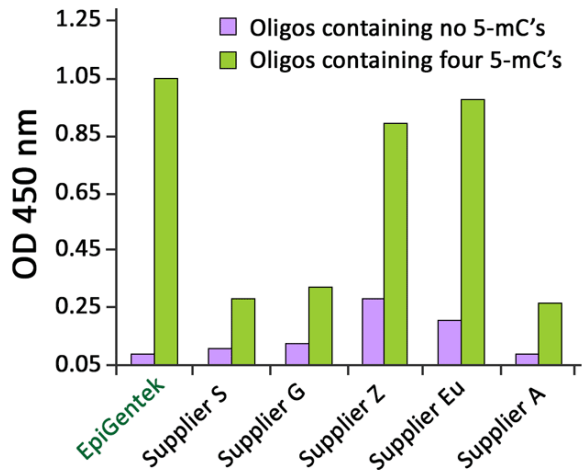
▲ Methylated DNA successfully captured by 5-methylcytosine antibody (anti-5mc) clone 33D3 during MeDIP. Fig. 2. Immunofluorescence staining with 5-methylcytosine antibody (anti-5mc) clone 33D3.



▲ Immunofluorescence staining with 5-methylcytosine antibody (anti-5mc) clone 33D3.



▲ Synthesized oligos containing different numbers of 5-methylcytosines were captured with the Clone 33D3 anti-5-methylcytosine antibody (Cat No. A-1014) and then colorimetrically detected. The results show that the oligos containing as few as two 5-mCs can still be captured and oligos with four or more 5-mCs can be fully captured by the antibody.



▲ Synthesized oligos containing different numbers of 5-methylcytosines were captured with anti-5-methylcytosine antibodies from various companies and then colorimetrically detected for comparison. Results show EpiGentek's 5-mC antibody has the highest sensitivity and specificity in capturing methylated DNA fragments.

## 5-Hydroxymethylcytosine (5-hmC) Monoclonal Antibody [HMC/4D9] Antibody (Component Cat. #C20000-1-5HMC4)

### Background

5-hydroxymethylcytosine (5-hmC), as a sixth DNA base with functions in transcription regulation, has been detected to be abundant in human and mouse brain and embryonic stem (ES) cells. In mammals, it can be generated by oxidation of 5-methylcytosine (5-mC), a reaction mediated by the ten-eleven translocation (TET) family of 5-mC hydroxylases. 5-hmC was demonstrated to play an important and different role than 5-mC in the regulation of DNA methylation, chromatin remodeling, and gene expression.

### Concentration

1 mg/ml

### Description

Mouse monoclonal antibody to 5-Hydroxymethylcytosine (5-hmC), clone HMC/4D9

### Specificity

Modified base 5-hydroxymethylcytosine (5-hmC), a broad range of species

### Isotype

IgG1

### Formulation

10 mM phosphate buffer, 150 mM NaCl, pH 7.4

### Storage

4°C. For long-term storage, aliquot and store at -20°C. Avoid repeated freezing and thawing. Multiple freeze/thaw cycles may result in decreased performance. Stable for 6 months from the date of shipment.

### Purity

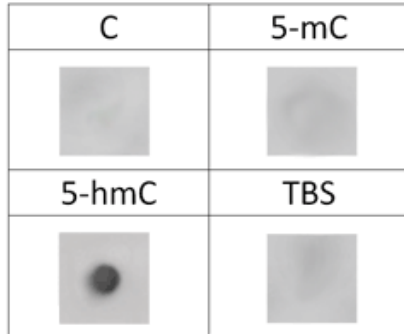
Protein A purified

### Alternative Names

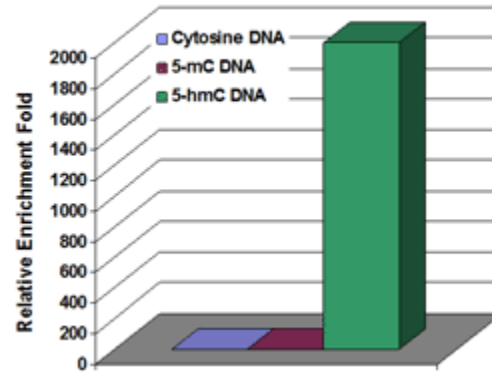
5-hydroxymethylcytidine, anti-5-hydroxymethylcytidine, anti-5-hydroxymethylcytosine, anti-5hmC, anti-5-hmC, anti-5-hmeC, anti-5hmeC, 5hmC, 5hmeC, 5-hmeC

### Application

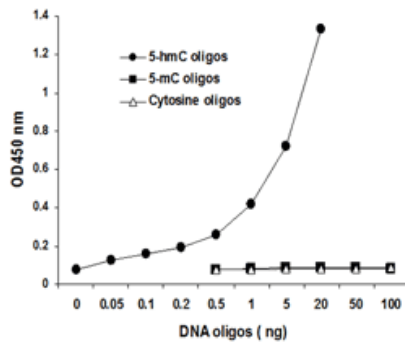
Immunofluorescence: 1:200 – 1: 500; Immunohistochemistry: 1:200 – 1: 500, ELISA: 1:1000 – 1:2000; Dot Blot: 1:2000; hMeDIP: 0.5-1 µg/10<sup>6</sup> Cells



▲ Dot blot analysis of A-1018 with DNA oligos containing cytosine (C), 5-methylcytosine (5mC), or 5-hydroxymethylcytosine (5hmC). 10 ng of C, 5mC, and 5hmC DNA oligos each were spotted on a membrane. The membrane was incubated with 0.5 µg/ml of Epigentek's 5hmC Clone HMC/4D9 antibody (1:2000 dilution), then with peroxidase conjugated goat anti-mouse IgG (dilution 1:3000). Specific signal was only observed with 5-hmC DNA oligos. Fig. 2. Demonstration of high sensitivity and specificity of Epigentek's 5-hmC Clone HMC/4D9 antibody against 5-hmC DNA. Synthetic DNA oligos containing 5-hmC, 5-mC or only cytosine, respectively were added into the assay wells at different concentrations and then specific signals was only detected with 5-hmC DNA.



▲ Selective enrichment of hydroxymethylated DNA. 50 pg of unmethylated, methylated, and hydroxymethylated DNA oligos were each spiked into fragmented human genomic DNA (500 ng). hMeDIP was processed with Epigentek's 5-hmC Clone HMC/4D9 antibody. Eluted DNA was analyzed by real time PCR with the primers to detect the presence of spiked DNA oligos. Fold-enrichment represents the amount of recovered DNA oligos and was calculated based on the Cts.



▲ Demonstration of high sensitivity and specificity of Epigentek's 5-hmC Clone HMC/4D9 antibody against 5-hmC DNA. Synthetic DNA oligos containing 5-hmC, 5-mC or only cytosine, respectively were added into the assay wells at different concentrations and then specific signals was only detected with 5-hmC DNA.

## HRP- Goat Anti-Mouse Secondary Antibody

(Component Cat. #C20000-1-HGM2)

### Description

Goat anti-mouse IgG recognizes mouse IgG whole molecule. This secondary antibody was purified using antigen affinity chromatography. The antibody is conjugated with Peroxidase.

### Antibody Type

Polyclonal Antibody

### Purification

Liquid; This product was prepared from monospecific antiserum by immunoaffinity chromatography, followed by solid phase adsorption(s) to remove any unwanted reactivities.

### Immunogen

Mouse IgG whole molecule

### Isotype

IgG

### Formulation

In 10 mM sodium phosphate, 75 mM NaCl, 50% (v/v) glycerol, pH 7.2

### Specificity

Mouse

### Storage

Store at 4°C. For long term storage store at -20°C. Aliquot to avoid repeated freezing and thawing.

### Handling Recommendations

The optimal working dilution should be determined by the end user. For maximum recovery of the products, centrifuge the vial prior to opening the cap.

### Applications & Suggested Dilutions

Western Blot: 1:1000-1: 10000; Immunohistochemistry: 1:100-1:500; Immunofluorescence: 1:100-1:500; ELISA: 1:2000-1:20000

## RELATED PRODUCTS

### Histone Modification Antibodies

A-1014	5-Methylcytosine (5-mC) Monoclonal Antibody [33D3]
A-1018	5HMC4 5-Hydroxymethylcytosine (5-hmC) Monoclonal Antibody [HMC/4D9]
A12003	HRP-Goat Anti-Mouse Secondary Antibody

### Histone Modification Panel Packs

C10000	Histone H3 Methylation Antibody Panel Pack I – Active Genes
C10001	Histone H3 Methylation Antibody Panel Pack I – Repression Genes
C10002	Histone H3 Methylation Antibody Panel Pack II – Active Genes
C10003	Histone H3 Methylation Antibody Panel Pack II – Repression Genes
C10004	Histone H3 Methylation Antibody Panel Pack III – Active Genes
C10005	Histone H3K4 Methylation Antibody Panel Pack
C10006	Histone H3K9 Methylation Antibody Panel Pack
C10007	Histone H3K27 Methylation Antibody Panel Pack
C10008	Histone H3K36 Methylation Antibody Panel Pack
C10009	Histone H3K79 Methylation Antibody Panel Pack
C10010	Histone H3 Acetylation Antibody Panel Pack I
C10011	Histone H3 Acetylation Antibody Panel Pack II
C10012	Histone H4K20 Methylation Antibody Panel Pack
C10013	Histone H4 Acetylation Antibody Panel Pack
C10014	Histone H3 Phosphorylation Antibody Panel Pack
C10015	Histone H3R2 Methylation Antibody Panel Pack
C10016	Histone H3R8 Methylation Antibody Panel Pack
C10017	Histone H3R17 Methylation Antibody Panel Pack
C10018	Histone H3R26 Methylation Antibody Panel Pack
C10019	Histone H4R3 Methylation Antibody Panel Pack