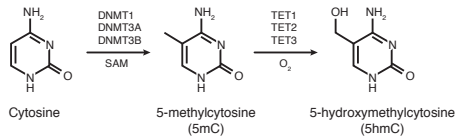
Methyl Hunter

MBL

For Research Use Only

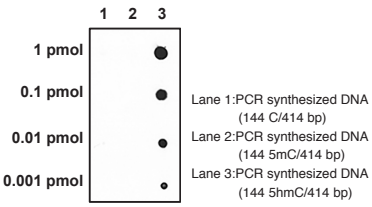
Anti-5-hydroxymethylcytosine (5hmC) pAb

5-hydroxymethylcytosine (5hmC) is a critical, epigenetic DNA modification generated from oxidation of 5-methylcytosine (5mC) by the enzymatic activity of TET family proteins. Though 5hmC is an intermediate metabolite of demethylation, it has been suggested that 5hmC itself is involved in self-renewal of stem cells, and is related to diseases such as neuronal disorders (Alzheimer's disease) and cancer. Elucidation of the functions of 5hmC and its relationship to diseases is expected to lead to the development of new therapeutic drugs. The development of new technologies is also ongoing in order to analyze the status of hydroxymethylcytosine modification in whole human genome.

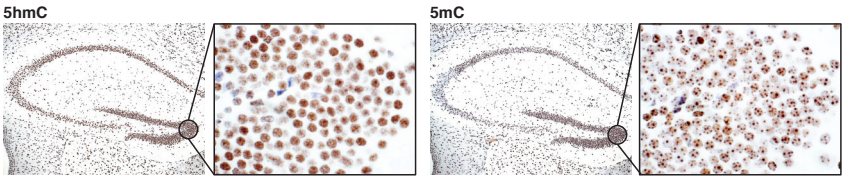


- Can be used for Dot blot, hMeDIP and Immunohistochemistry
- Allows efficient immunoprecipitation of DNA fragments containing 5hmC

Dot blotting



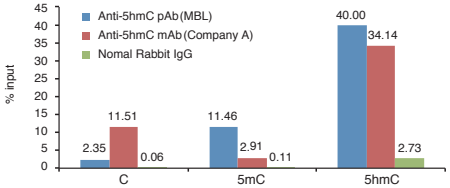
Immunohistochemistry



Tissues： Adult mouse brain (parrafin section)
Antigen retrieval: Heat-treated（95ºC, 40min）
Antibody： Anti-5hmC pAb (Code. PM077; 1:500), Anti-m^{5}C mAb* (Code. D346-3; 0.25 μg/mL）

＊This antibody cross-reacts with 5-methylcytosine in DNA.

Hydroxymethylated DNA Immunoprecipitation



Sample: Mouse Liver genomic DNA (1 μg)+PCR synthesized DNA (50 pg)
Antibody: Code. PM078, Antibody of Company A or Nomal Rabbit IgG

C: PCR synthesized DNA (13 CpG/136 bp)
5mC: PCR synthesized DNA (13 5mCpG/136 bp)
5hmC: PCR synthesized DNA (13 5hmCpG/136 bp)

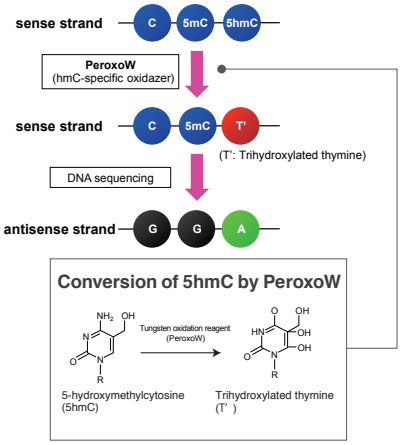
Code	Product	Size	Application	Isotype
PM077	Anti-5-hydroxymethylcytosine (5hmC) pAb	100 μL	Dot blot / DNA-IP / IH	Rabbit Ig (aff.)

5hmC detection Kit

Tungsten oxidative reagent (PeroxoW) specifically and effectively oxidizes 5hmC via conversion of 5hmC in DNA to trihydroxylated thymine (T'). In DNA sequence analysis using the oxidized DNA, T' is displayed as adenine (A) in the antisense strand. The existence of 5hmC and its position is easily identified by comparing the sequences of treated and non-treated DNA.

- Tungsten oxidative reagent allows quick and easy detection of 5hmC in DNA at the basal level.
- At least 30 cycles of tungsten oxidation are required to detect 5hmC. (Increasing the number of cycles as much as possible is recommended.)
- PCR amplification has been shown to be unsuccessful after treatment with the tungsten oxidative reagent. Detection may be difficult if the starting quantity of the sample is prohibitively low.

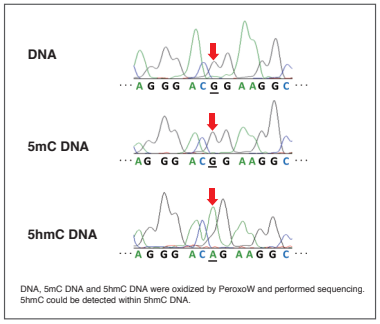
Detection of 5hmC by tungsten oxidative reagent



Kit components: 25 tests		
Oxidation reagent	Dinuclear Peroxotungsten (PeroxoW)	25 mg
Reaction buffer	2× 5hmC Oxidation Buffer	625 μL
Control DNA	Synthesized double-stranded DNA, 100 bp, 100 ng/μL	25 μL
Control sequencing primer	Sequencing primer (Reverse), 2 μM	25 μL

Code	Product	Size
5350	MethylHunter 5hmC detection kit	25 tests

[Reference] Okamoto A *et al. Chem Commun (Camb).* 47, 11231-3 (2011)



Sequencing anaylysis of 5hmC DNA

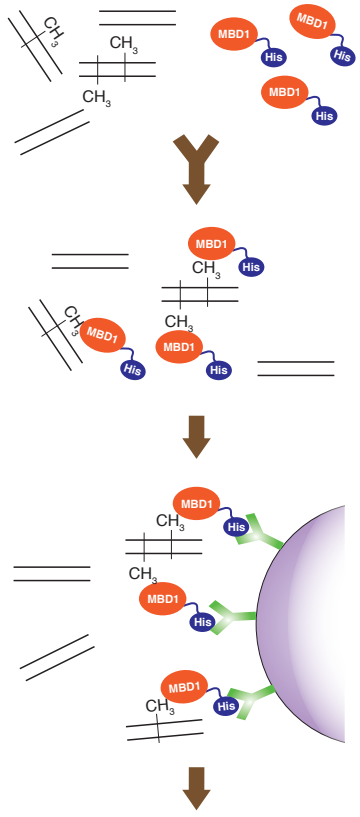
MEDICAL & BIOLOGICAL LABORATORIES CO., LTD.
http://ruo.mbl.co.jp/

MBD1-based Methylated DNA Enrichment Kit 2

MBD1 is capable of enriching methylated DNA at exceptionally high specificity and allows detection from 1 ng of input DNA by real-time PCR.

- Methylated DNA enrichment kit that uses the MBD1 protein
- Higher yield! Higher enrichment rate compared with antibody-based enrichment methods or other MBD protein-based methods
- Anti-His tag antibody magnetic beads and DNA extraction reagents included in the kit

Procedure summary



Fragmented genomic DNA and MBD1 (His-tag) are mixed together.

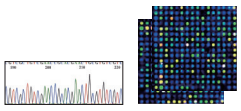
MBD1 binds specifically methylated CpG DNA. With a larger number of methylated sites, MBD1 binds more efficiently, and the efficiency of enrichment increases.

Only methylated DNA that binds to MBD1 is extracted using magnetic beads conjugated anti-His-tag antibodies.

Analysis of methylated DNA

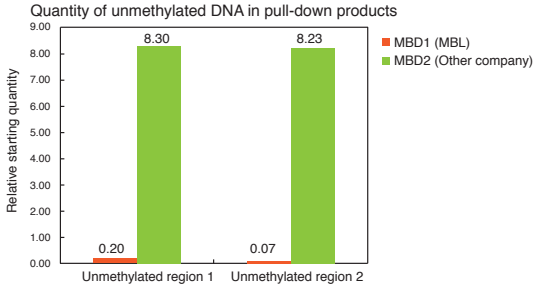
- **Microarray**
- **Real-time RCR**
- **Next generation sequencing**

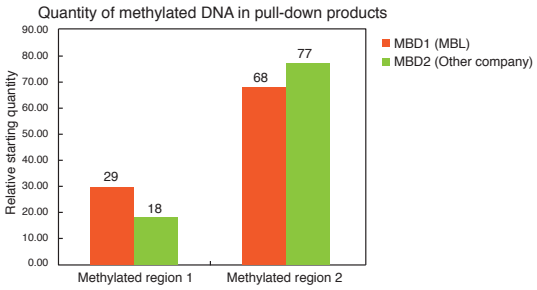
Enriched methylated DNA can be used in various analyses.



High specificity of MBD1 for methylated DNA

Comparison of MBD1 and MBD2 with regards to the quantity of methylated and unmethylated DNA contained in pull-down products

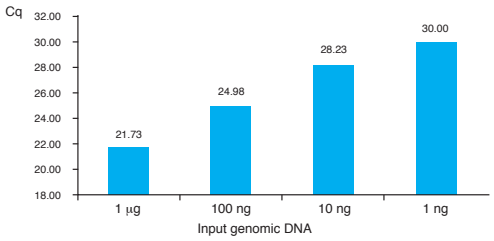




Input: 5 μg of control DNA
Standard: input DNA 10 ng, 1 ng, 0.1 ng/template
Methylated region: 1:40 diluted pull-down DNA 1 μL/template
Unmethylated region: 1:2 diluted pull-down DNA 1 μL/template

The quantity of unmethylated DNA in pull-down products with MBD1 was about 40- to 100-fold less than that of products with MBD2.
MBD1 is capable of enriching methylated DNA at extremely high specificity.

Quantity of input DNA that can be enriched with MBD1



The MBD1 kit was used to enrich methylated DNA obtained from each input of genomic DNA from mouse liver, and real-time PCR was performed using control primers. Amplification and enrichment of the methylated DNA were confirmed with ≥ 1 ng of input DNA.

Code	Product	Size	Storage temp.
5275-100	MethylHunter MBD1-based Methylated DNA Enrichment Kit 2	25 tests	-20°C
5275-200			4°C

* Please note that 5275-100 and 5275-200 are sold together but should be stored at different temperatures.
* Inexpensive kits without magnetic beads and DNA extraction reagents are also available (Code: 5270-100/5270-200).

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Produced by
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