For Research Use Only.
Not for use in diagnostic procedures.

Anti-5-hydroxymethylcytosine (5hmC) mAb

CODE No.	M218-3
CLONALITY	Monoclonal
CLONE	1G10
ISOTYPE	Rabbit IgG
QUANTITY	100 µL, 1 mg/mL
SOURCE	Isolated from phage display libraries using immunized rabbit spleen. Purified IgG from culture supernatant of stable CHO cell clone.
IMMUNOGEN	BSA-conjugated 5-hydroxymethylcytidine
FORMURATION	PBS containing 50% Glycerol (pH 7.2). No preservative is contained.
STORAGE	This antibody solution is stable for one year from the date of purchase when stored at -20°C.

APPLICATIONS-CONFIRMED

Hydroxymethylated DNA immunoprecipitation (hMeDIP)	1.0 µg/ mL
Dot blotting	0.2 µg/ mL

APPLICATION-UNDER EVALUATION

Immunohistochemistry	Can be used. (0.02 µg/mL)

For more information, please visit our web site **http://ruo.mbl.co.jp/**

MEDICAL & BIOLOGICAL LABORATORIES CO., LTD.
URL http://ruo.mbl.co.jp/
e-mail support@mbl.co.jp, TEL 052-238-1904

RELATED PRODUCTS

Antibodies

M218-3	Anti-5-hydroxymethylcytosine (5hmC) mAb
PM077	Anti-5-hydroxymethylcytosine (5hmC) pAb
MI-11-3	Anti-Bromodeoxyuridine mAb (2B1)
MI-11-5	Anti-Bromodeoxyuridine mAb-PE (2B1)
D209-3	Anti-Histone H1 mAb (C14093)
D210-3	Anti-Histone H2A mAb (C10037)
D212-3	Anti-Histone H2B mAb (C14264)
D345-3	Anti-1-methyladenosine (m^1A) mAb (AMA-2)
D346-3	Anti-5-methylcytidine (m^5C) mAb (FMC-9)
PM006	Anti-Phospho-Histone H3 (Ser28) (Human) pAb
PM006-A48	Anti-Phospho-Histone H3 (Ser28) (Human) pAb -Alexa Fluor® 488
RN011M	Anti-2,2,7-trimethylguanosine (m3G/TMG) mAb
CY-M1029	Anti-Acetylated Histone/p53 (Lys382) mAb (TM-5C5)
CY-P1011	Anti-HDAC1 (Histone Deacetylase 1) pAb
CY-P1012	Anti-HDAC2 (Histone Deacetylase 2) pAb
CY-P1015	Anti-Phospho-Histone-H2A.X (Ser139) pAb
PM035	Normal Rabbit IgG

Kits

5350	MethylHunter 5hmC detection kit
5270-100	MethylHunter MBD1-based Methylated DNA Enrichment Kit
5275-100	MethylHunter MBD1-based Methylated DNA Enrichment Kit 2
MEX-E	ExoCap™ Nucleic Acid Elution Buffer

Other related antibodies and kits are also available.
Please visit our website at http://ruo.mbl.co.jp/

Hydroxymethylated DNA Immunoprecipitation (hMeDIP)

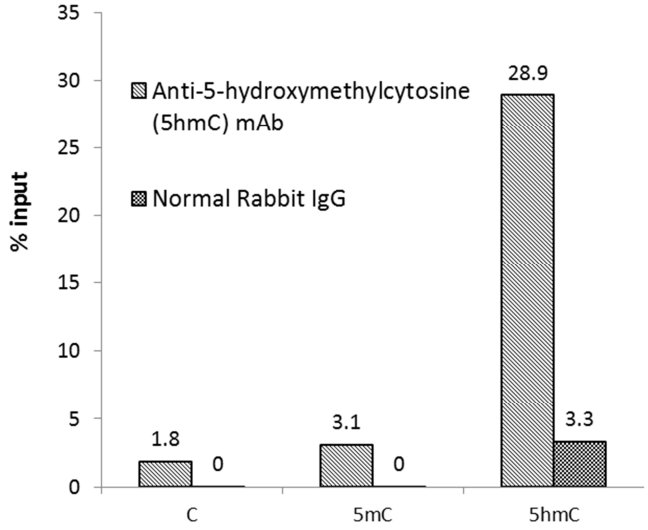
1) Sample preparation:
   a) Prepare 1 µg of genomic DNA fragment by appropriate method.
   b) Add 50 pg of PCR synthesized C-, 5mC- or 5hmC-containing DNA as a spike-in control.
   c) Dissolve the DNA sample in TE buffer [10 mM Tris-HCl (pH 7.4), 1 mM EDTA] and adjust the volume to 20 µL.
   d) Heat the DNA sample at 99°C for 10 min., then quench at 0°C for 10 min.
2) Add 430 µL of TE buffer, 50 µL of 10 x IP buffer [100 mM Na-Phosphate (pH 7.0), 1.4 M NaCl, 0.5 % Triton X-100] and Anti-5-hydroxymethylcytosine (5hmC) mAb (M218-3) as suggested in the **APPLICATIONS** or 0.5 µg of Normal Rabbit IgG (MBL; code no. PM035). Incubate with gentle agitation for 2 hr. at 4°C.
3) During step 2), wash 40 µL of Dynabeads® M-280 Sheep anti-Rabbit IgG (Life Technologies; code no. 11203D) with 800 µL of PBS and place the tube on the magnetic rack (MBL; Code no. 3190) for a few seconds. Discard the supernatant carefully. Resuspend the beads with 50 µL of 1 x IP buffer [10 mM Na-Phosphate (pH 7.0), 140 mM NaCl, 0.05 % Triton X-100].
4) Add 50 µL of washed beads suspension (prepared in step 3)) to DNA and antibody mixture. Incubate with gentle agitation for 2 hr. at 4°C.
5) Place the tube on the magnetic rack for a few seconds and discard the supernatant carefully.
6) For washing the beads, add 700 µL of 1 x IP buffer and incubate the tube with gentle rotation for 10 min. at room temperature.
7) Place the tube on the magnetic rack for a few seconds and discard the supernatant carefully.
8) Repeat 3 times steps 6) - 7).
9) Isolate nucleic acids in the following methods.

[DNA isolation; 2-step method in ExoCap™ Nucleic Acid Elution Buffer (MBL; code no. MEX-E)]

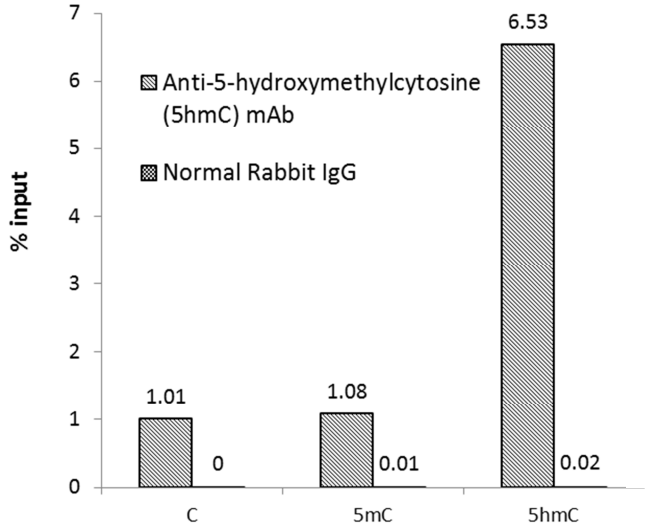
1) Prepare Master mix solution by diluting 10 µL of Nucleic Acid Elution Buffer 1 with 240 µL of Nucleic Acid Elution Buffer 2 per sample.
2) Add 250 µL of Master mix solution to the washed magnetic beads, vortex thoroughly and spin-down.
3) Add 150 µL of Nucleic Acid Elution Buffer 3 to each tube, vortex thoroughly and spin-down.
4) Dispense 2 µL of Nucleic Acid Elution Buffer 4 to each new microcentrifuge tube for step 6).
5) Place the tube on a magnetic stand to separate the beads from the solution.
6) After the solution becomes clear (about 1 min), carefully transfer the supernatant to the tube prepared in step 4).
7) Add 400 µL of 100% ethanol to each tube, vortex briefly but thoroughly, and spin-down.
8) Incubate the tube at -20ºC or below for 20 min (or overnight, if necessary).
9) Centrifuge the tube at 12,000 × *g* for 10 min. at 4ºC, and add 2 µL of Nucleic Acid Elution Buffer 4.
10) Add 400 µL of 100% ethanol to each tube, vortex briefly but thoroughly, and spin-down.
11) Incubate the tube at -20ºC or below for 20 min (or for overnight, if necessary).
12) Centrifuge the tube at 12,000 × *g* for 10 min at 4ºC, and aspirate the supernatant carefully.
13) Rinse the pellet with 500 µL of ice-cold 70% ethanol, and mix briefly.
14) Centrifuge the tube at 12,000 × *g* for 3 min at 4ºC, and aspirate the supernatant carefully.
15) Repeat steps 13) – 14) to rinse the pellet once again.
16) Aspirate the excess ethanol, and leave the tube lids open for 5-15 min. at room temperature to evaporate the remaining ethanol.
17) Reconstitute the pellet in 60 µL of nuclease-free water.
18) Store at -80ºC until starting following analysis.

[DNA isolation; Alternative method]

1) Add 250 µL of Proteinase K digestion buffer [50 mM Tris (pH 8.0), 10 mM EDTA, 0.5 % SDS]. Incubate for 1 hr. at 50°C with inversion every 10 min.
2) Perform DNA extraction by Phenol/Chloroform extraction followed by ethanol precipitation.
3) Dissolve the pellet in 60 µL of nuclease-free water.



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



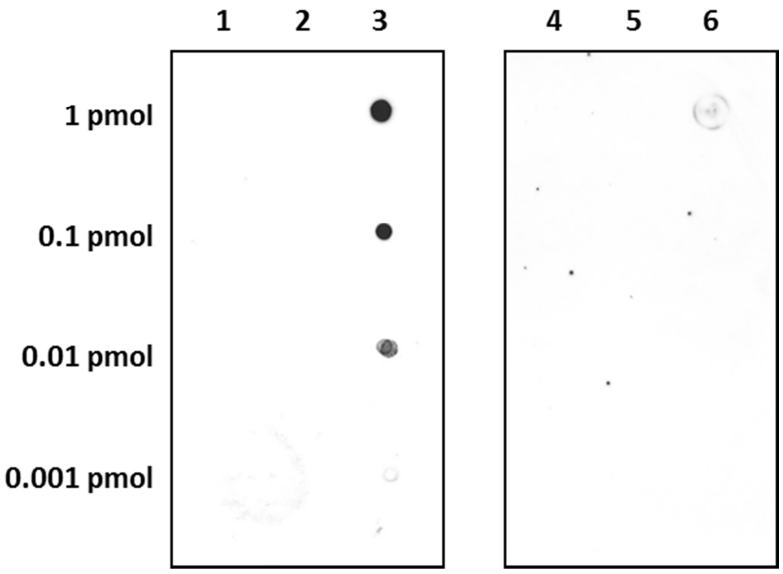
C: Synthesized ssDNA (15 CpG/100 nt)
5mC: Synthesized ssDNA (1 5mCpG/100 nt)
5hmC: Synthesized ssDNA (1 5hmCpG/100 nt)

Analysis of immnoprecipitated DNA with Real-time PCR using spike-in control specific primers

Dot blotting

1) Sample preparation:
   a) Prepare DNA samples by appropriate method (e.g., 5hmC-containing DNA by performing PCR).
   b) Add 0.1 volumes of 1 M NaOH to the DNA samples.
   c) Heat the DNA samples at 99ºC for 5 min., then quench at 0ºC for 5 min.
   d) Add 0.1 volumes of 6.6 M NH4OAc to the DNA samples.
2) Blot 1 µL of different concentrations of DNA samples onto a nitrocellulose membrane.
3) Cross-link the DNA samples using UV illuminator for 5 min.
4) To reduce nonspecific binding, soak the membrane in 5% skimmed milk (in PBS, pH 7.2) for 1 hr. at room temperature.
5) Wash the membrane with PBS-T [0.05% Tween-20 in PBS] (5 min. x 3 times).
6) Incubate the membrane with primary antibody diluted with 1% skimmed milk (in PBS, pH 7.2) as suggested in the **APPLICATIONS** for 1 hr. at room temperature. (The concentration of antibody will depend on the conditions.)
7) Wash the membrane with PBS-T (5 min. x 3 times).
8) Incubate the membrane with the 1:5,000 Anti-IgG (Rabbit) pAb-HRP (MBL; code no. 458) diluted with 1% skimmed milk (in PBS, pH 7.2) for 1 hr. at room temperature.
9) Wash the membrane with PBS-T (5 min. x 3 times)
10) Wipe excess buffer on the membrane, then incubate it with appropriate chemiluminescence reagent for 1 min. Remove extra reagent from the membrane by dabbing with paper towel, and seal it in plastic wrap.
11) Expose to an X-ray film in a dark room for 1 min~30 min. Develop the film as usual. The condition for exposure and development may vary.

(Positive control for Dot blotting; PCR synthesized DNA containing 5hmC)



Dot blot analysis of 5hmC-containing DNA

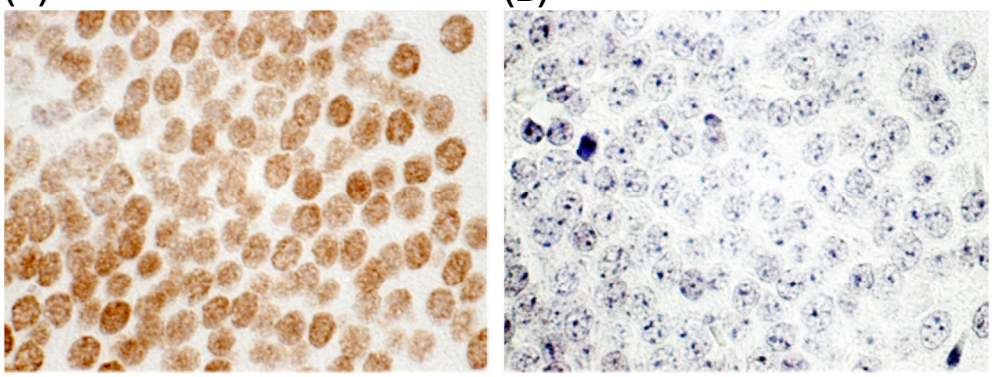
Lane 1: PCR synthesized DNA containing 114 C residues (414 bp)
Lane 2: PCR synthesized DNA containing 114 5mC residues (414 bp)
Lane 3: PCR synthesized DNA containing 114 5hmC residues (414 bp)

Lane 4: Synthesized ssDNA containing 15 C residues (100 nt)
Lane 5: Synthesized ssDNA containing 1 5mC residue (100 nt)
Lane 6: Synthesized ssDNA containing 1 5hmC residue (100 nt)

Immunoblotted with Anti-5-hydroxymethylcytosine (5hmC) mAb (M218-3)

Immunohistochemstry (paraffin section)

(A) **(B)**



Immunohistochemical detection of 5hmC-containing DNA in mouse hippocampus

(A): Stained with M218-3
(B): Stained with M218-3 pre-treated with antigen

Antigen retrieval: Heat-treated (95ºC, 40 min)/10 mM Citrate buffer (pH 6.0)
Incubation: For 50 min. at room temperature

Brown: Anti-5-hydroxymethylcytosine (5hmC) mAb (M218-3)
Blue: Hematoxylin