9853 Pacific Heights Blvd.
Suite D. San Diego,
CA 92121, USA
Tel: 858-263-4982
Email: info@abeomics.com

10-9585: Recombinant Rabbit Monoclonal Antibody to 5-methylcytosine (5-mC)(Clone: RM23)(Discontinued)

Clonality :	Monoclonal
Clone Name :	RM231
Application :	ELISA,IHC,MeDIP,DB
Reactivity :	Human
Format :	Purified
Isotype :	Rabbit IgG
Immunogen Information :	BSA-conjugated 5-methylcytosine.

Product Info

Amount :	50 µg
Purification :	Protein A affinity purified from an animal origin-free culture supernatant
Content :	1 mg/ml in 50% Glycerol/PBS with 1% BSA and 0.09% sodium azide
Storage condition :	Store at -20°C. Avoid repeated freeze and thaw cycles.

Application Note

Clone RM231 reacts to 5-methylcytosine in both single-stranded and double-stranded DNA. No cross reactivity with non-methylated cytosine and hydroxymethylcytosine in DNA. Dot Blot: 0.5 µg/ml Ã□â□□ 2 µg/ml; ELISA: 0.1 µg/ml - 1 µg/ml; ICC: 0.5 µg/ml - 2 µg/ml; MeDIP: 0.2 µg/ml- 2 µg/ml.

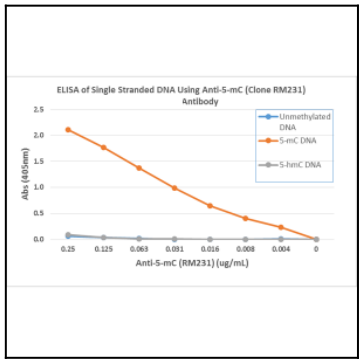

Figure 1: ELISA of single stranded DNA using rabbit monoclonal anti-5-mC (Clone: RM231) antibody. The plate was coated with streptavidin and then biotinylated single stranded unmethylated DNA, 5-Methylcytosine (5-mC) DNA, and 5-Hydroxymethylcytosine (5-hmC) DNA. A serial dilution of Clone: RM231 was used as the primary antibody, and an alkaline phosphatase conjugated anti-rabbit IgG as the secondary antibody.

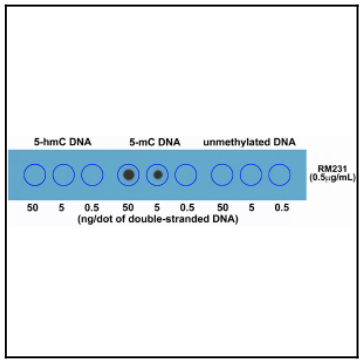

Figure 2: Dot blot of double stranded DNA using rabbit monoclonal anti-5-mC (Clone: RM231) antibody. The membrane waspre-spotted with 50, 5, and 0.5 ng/dot of double stranded 5-Hydroxymethylcytosine (5-hmC) DNA, 5-Methylcytosine (5-mC) DNA, and unmethylated DNA. The pre-spotted membrane was then blotted with Clone: RM231.

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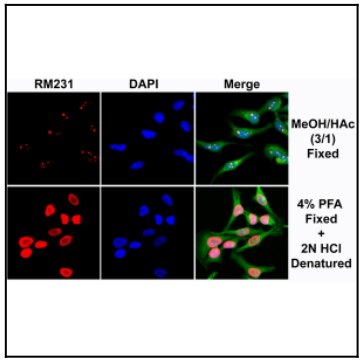

Figure 3: Immunocytochemical staining of HeLa cells using rabbit monoclonal anti-5-mC (Clone: RM231) antibody (red). Actin filaments have been labeled with fluorescein phalloidin (green), and nuclei stained with DAPI (blue).

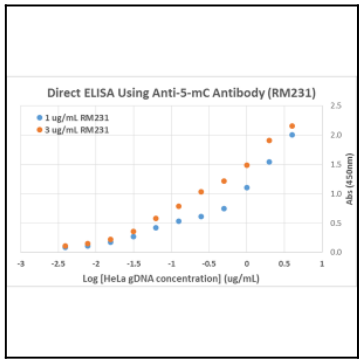

Figure 4: Direct ELISA of HeLa cell genomic DNA using anti-5-mC antibody (RM231). The plate was directly coated with different concentrations of genomic DNA isolated from HeLa cells. 1 µg/ml or 3 µg/ml of RM231 was used as the primary antibody, and a HRP conjugated anti-rabbit IgG as the secondary antibody.

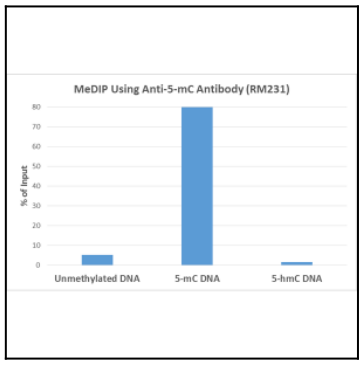

Figure 5: MeDIP was performed using anti-5-mC antibody (Clone: RM231) at a 2:1 DNA:Ab ratio. 1 ng of unmethylated, 5-Methylcytosine (5-mC) or 5-Hydroxymethylcytosine (5-hmC) DNA standard (897 bp) was spiked in 1ug of genomic DNA isolated from HeLa cells as the control. Realtime PCR was then performed to determine the capture of DNA standard as in % of input.