

10-9586: Recombinant Rabbit Monoclonal Antibody to 5-hydroxymethylcytosine (5-hmC); 5'-hmC (Clone: RM236)(Discontinued)

Clonality :	Monoclonal
Clone Name :	RM236
Application :	ELISA,IHC,MeDIP,DB
Reactivity :	Human
Format :	Purified
Isotype :	Rabbit IgG
Immunogen Information :	BSA-conjugated 5-hydroxymethylcytosine.

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 RM236 reacts to 5-hydroxymethylcytosine in both single-stranded and double-stranded DNA. No cross reactivity with non-methylated cytosine and methylcytosine in DNA. Dot Blot: 0.2 µg/ml – 1 µg/ml; ELISA: 0.1 µg/ml - 1 µg/ml; IHC: 0.1 µg/ml - 1 µg/ml; ICC: 0.5 µg/ml- 2 µg/ml; hMeDIP: 0.2 µg/ml- 2 µg/ml.

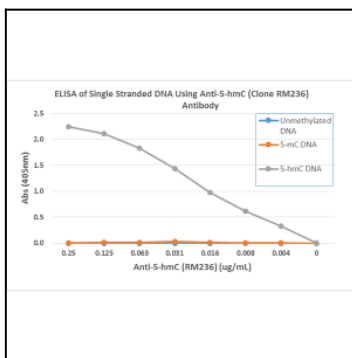


Figure 1: ELISA of single stranded DNA using rabbit monoclonal anti-5-hmC (Clone: RM236) antibody. The plate was coated with streptavidin and then biotinylated single stranded unmethylated DNA, 5-Methylcytosine (5-mC) DNA, and 5-Hydroxymethylcytosine (5-hmC) DNA. A serial dilution of Clone: RM236 was used as the primary antibody, and an alkaline phosphatase conjugated anti-rabbit IgG as the secondary antibody.

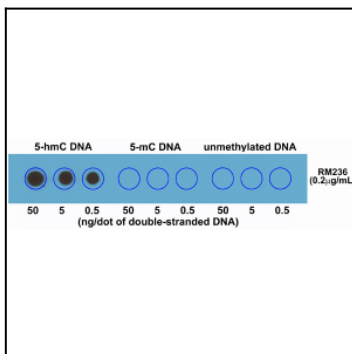


Figure 2: Dot blot of double stranded DNA using rabbit monoclonal anti-5-hmC (Clone: RM236) antibody. The membrane was pre-spotted with 50, 5, and 0.5 ng/dot of double stranded 5-Hydroxymethylcytosine (5-hmC) DNA, 5-Methylcytosine (5-mC) DNA, and unmethylated DNA. The pre-spotted membrane was then blotted with Clone: RM236.

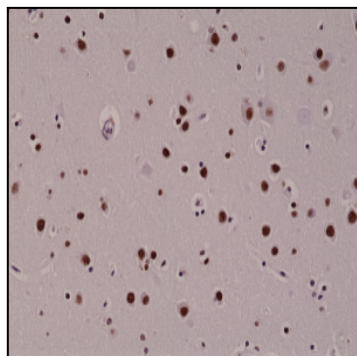


Figure 3: Immunohistochemical staining of formalin fixed and paraffin embedded human brain tissue sections, using rabbit monoclonal anti-5-hmC (Clone: RM236) antibody.

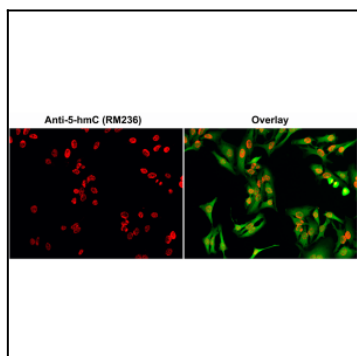


Figure 4: Immunocytochemical staining of HeLa cells using 0.5 µg/ml anti-5-hmC antibody (Clone: RM236) (red). Actinfilaments was labeled with fluorescein phalloidin (green). HeLa cells were fixed with 4% parafor- maldehyde and peClone: RMeabilized with methanol (20°C) before treatment with 2 N HCl for 30 min at 37 °C to denature DNA.

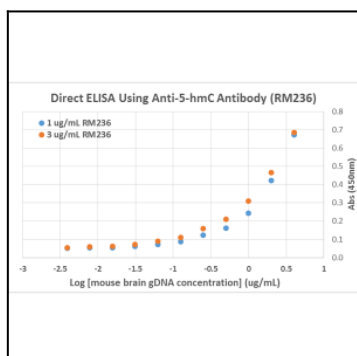


Figure 5: Direct ELISA of mouse brain genomic DNA using anti-5-hmC antibody (Clone: RM236). The plate was directly coated with different concentrations of genomic DNA isolated from mouse brain tissue. 1 µg/ml or 3 µg/ml of Clone: RM236 was used as the primary antibody, and a HRP conjugated anti-rabbit IgG as the secondary antibody.

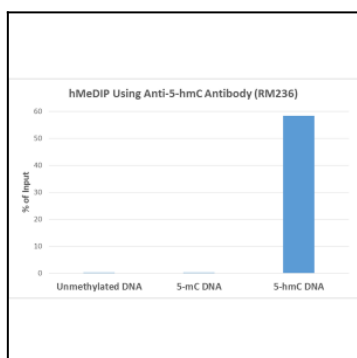


Figure 6: hMeDIP was performed using anti-5-hmC antibody (Clone: RM236) at a 10:1 DNA:Ab ratio. 1 ng of unmethylated, 5-Methylcytosine (5-mC) or 5-Hydroxymethylcytosine (5-hmC) DNA standard (897 bp) was spiked in 1ug of genomic DNA isolated from HeLa cells as the control. Realtime PCR was then performed to determine the capture of DNA standard as in % of input.