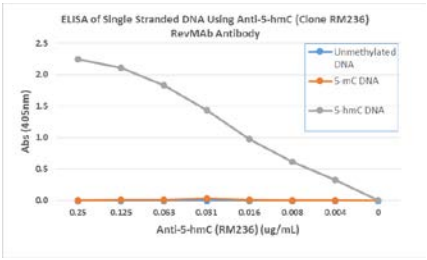
RevMAb Biosciences
www.revmab.com

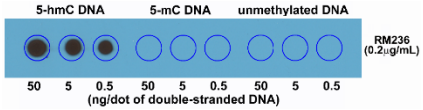
RevMAb Biosciences USA, Inc.
830 Dubuque Ave, South San Francisco, CA 94080, USA

Certificate of Analysis

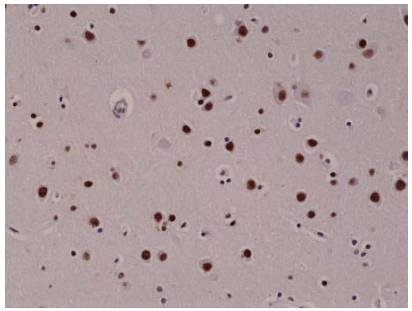
Product:	Rabbit Monoclonal Antibody Anti-5- hydroxymethylcytosine Rabbit Monoclonal Antibody, Clone RM236
Catalog No.:	31-1111-00
Lot No.:	
Clone	RM236
Specificity	This antibody reacts to 5-hydroxymethylcytosine in both single-stranded and double-stranded DNA. No cross reactivity with non-methylated cytosine and methylcytosine in DNA.
Application:	hMeDIP, ELISA, Dot Blot, Immunocytochemistry, Immunohistochemistry, Flow Cytometry
Immunogen:	BSA-conjugated 5-hydroxymethylcytosine.
Purity:	Protein A affinity purified from an animal origin–free culture supernatant
Size:	50 µg
Concentration:	1.0 mg/mL
Buffer:	50% Glycerol/PBS with 1% BSA and 0.09% sodium azide
Usage:	Dot Blot: 0.2 µg/mL - 1 µg/mL; IHC: 0.1 µg/mL - 1 µg/mL; ; ICC: 0.5 µg/mL - 2 µg/mL; ELISA: 0.1µg/mL - 1 µg/mL.; hMeDIP: 0.2 µg/mL - 2 µg/mL; FC: 1 µg/mL - 5 µg/mL.
Storage and Stability:	Stable for 1 Year at -20.0°C from date of receipt.
Country of Origin:	U.S.A.
Intended Use:	**For Research Use Only Not for Diagnostic or Therapeutic Use**



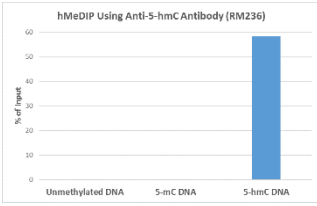
ELISA of single stranded DNA using anti-5-hmC antibody (RM236). The plate was coated with streptavidin and then biotinylated single stranded unmethylated DNA, 5-Methylcytosine (5-mC) DNA, and 5-Hydroxymethylcytosine (5-hmC) DNA. A serial dilution of RM236 was used as the primary antibody, and an alkaline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



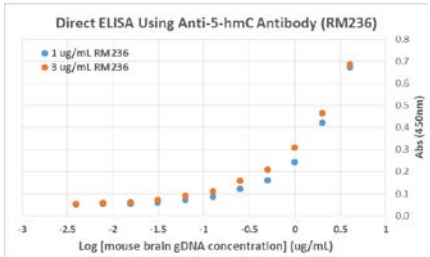
Dot blot of double stranded DNA using anti-5-hmC antibody (RM236) antibody. The membrane was pre-spotted with 50, 5, and 0.5 ng/dot of double stranded 5-Hydroxymethylcytosine (5-hmC) DNA, 5-Methylcytosine (5-mC) DNA, and unmethylated DNA.



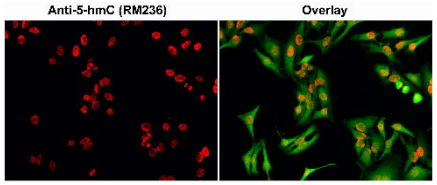
Immunohistochemical staining of formalin fixed and paraffin embedded human brain tissue sections, using rabbit monoclonal anti-5-hmC (clone RM236) antibody.



hMeDIP was performed using anti-5-hmC antibody (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.



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



Immunocytochemical staining of HeLa cells using 0.5µg/mL anti-5-hmC antibody (RM236) (red). Actin filaments was labeled with fluorescein phalloidin (green). HeLa cells were fixed with 4% paraformaldehyde and permeabilized with methanol (−20 °C) before treatment with 2 N HCl for 30 min at 37 °C to denature the DNA.