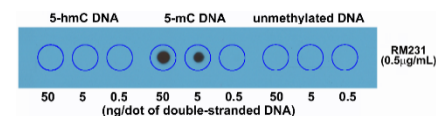
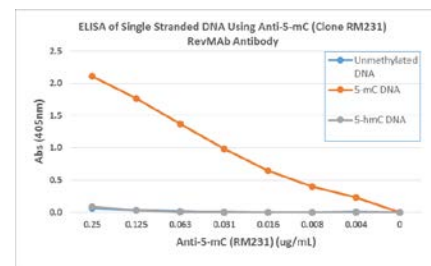


Certificate of Analysis

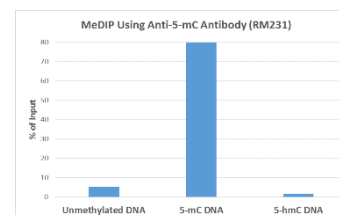
| | |
|-------------------------------|--|
| Product: | Rabbit Monoclonal Antibody Anti-5-Methylcytosine Rabbit Monoclonal Antibody, Clone RM231 |
| Catalog No.: | 31-1110-00 |
| Lot No.: | |
| Clone | RM231 |
| Specificity | This antibody reacts to 5-methylcytosine in both single-stranded and double-stranded DNA. No cross reactivity with non-methylated cytosine and hydroxymethylcytosine in DNA. |
| Application: | MeDIP, ELISA, Dot Blot, Immunocytochemistry, Immunohistochemistry, Flow Cytometry |
| Immunogen: | BSA-conjugated 5-methylcytosine. |
| 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: 0.5 µg/mL - 2 µg/mL; ICC: 0.5 µg/mL - 2 µg/mL; ELISA: 0.1µg/mL - 3 µg/mL.; MeDIP: 0.2 µg/mL - 2 µg/mL; FC: 0.5 µg/mL - 2 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 |



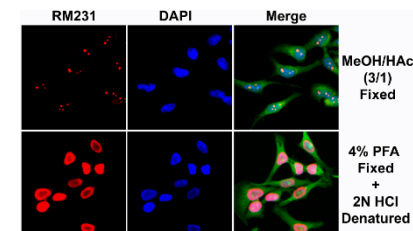
Dot blot of double stranded DNA using anti-5-mC antibody (RM231). The membrane was pre-spotted with 50, 5, and 0.5 ng/dot of double stranded 5-Hydroxymethylcytosine (5-hmC) DNA, 5-Methylcytosine (5-mC) DNA, and unmethylated DNA.



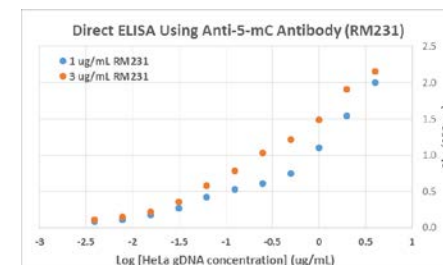
ELISA of single stranded DNA using anti-5-mC antibody (RM231). The plate was coated with streptavidin and then biotinylated single stranded unmethylated DNA, 5-Methylcytosine (5-mC) DNA, and 5-Hydroxymethylcytosine (5-hmC) DNA. A serial dilution of RM231 was used as the primary antibody, and an alkaline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



MeDIP was performed using anti-5-mC antibody (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.



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



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.