

Dot blot protocol for 5-hydroxymethylcytosine monoclonal antibody

(Cat. No. MAb-633HMC-050;MAb-633HMC-100)

- Prepare dilutions of normal C, 5-mC- and 5-hmC-containing PCR products from the "5-hmC, 5-mC & cytosine DNA standard pack" (as an alternative PCR products amplified with the Diagenode hmdCTP [Cat. No. AF-102-0250] and Methyl Taq polymerase [Cat. No. AF-103-0250] can also be used) in 2-5 μ l of 0.1M NaOH.

We recommend the use of 0.1, 0.5, 2 and 10 pmoles equivalent of C bases per dot for protocol optimization.

- Denature at 99°C for 5 minutes.
- Cool down on ice, spin down and neutralize with 0.1vol of 6.6M ammonium acetate.
- Spot on membrane (e.g., Amersham Hybond-N+) and air-dry.
- UV-x-link (1x 'auto cross-link').
- Block the membrane with Blocking Solution 10% milk, 1% BSA, PBT (1xPBS + 0.1%Tween-20) overnight at 4°C (as an alternative TBS (Tris Buffered Saline, pH7.5 Tris 50mM, NaCl 150mM) and Western Blocking reagent (Roche, Cat. No. 11921673001) can be used following manufacturer instructions).
- Incubate with 5-hmC rat monoclonal antibody (1:500 dilution; 4 μ g/ml final concentration) (Cat. No. MAb-633HMC-050 / 100) for maximum 1 hour in Blocking Solution at room temperature.
- Wash 3 to 4 times at room temperature with PBT for 30 minutes (or more if convenient).
- Incubate with HRP secondary antibody (1:10,000 in Blocking Solution) for 30 minutes to 60 minutes maximum at room temperature.
- Wash 3 to 4 times with PBT for 30 minutes or more if convenient.
- Detect with ECL Plus Western Blotting Reagent Pack (GE Healthcare, formerly Amersham Biosciences).

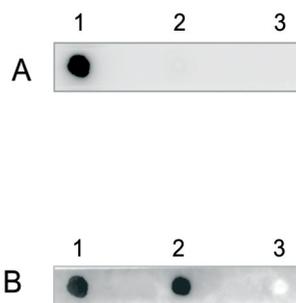


Figure 1
Dotblot analysis of the the C, mC and hmC PCR controls with the Diagenode 5-hmC and 5-mC monoclonal antibodies

10 pmol of C-bases equivalent (+/- 200 ng) of the hmC (1), mC (2) and C (3) PCR controls from the Diagenode "5-hmC, 5-mC & cytosine DNA Standard Pack" (Cat. No. AF-101-0020) were spotted on a membrane (Amersham Hybond-N+). The membrane was incubated with 5-hydroxymethylcytosine monoclonal antibody (rat) (Cat. No. MAb-633HMC-100) (dilution 1:500 ; 4 µg/ml final concentration) (panel A). Membranes were exposed during 20 seconds.

Incubation of the same membrane with the 5-methylcytosine monoclonal antibody (Cat. No. MAb-335MEC-100/500) (dilution 1:250) (panel B). Note that the membrane was not stripped after the 5-hmC incubation. The left spot represents the remaining hmC signal. This result confirms that an equal amount of mC bases was spotted at position 2.

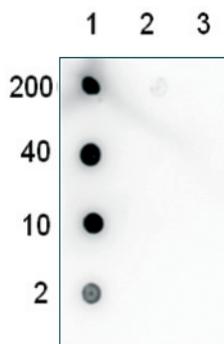


Figure 2
Dotblot analysis of the Diagenode 5-hmC rat monoclonal antibody with the C, mC and hmC PCR controls

200 to 2 ng (equivalent of 10 to 0.1 pmol of C-base) of the hmC (1), mC (2) and C (3) PCR controls from the Diagenode "5-hmC, 5-mC & cytosine DNA Standard Pack" (Cat. No. AF-101-0020) were spotted on a membrane (Amersham Hybond-N+). The membrane was incubated with 4 µg/ml (dilution 1:500) of the rat 5-hydroxymethylcytosine monoclonal antibody. The membranes were exposed for 30 seconds.