

PRODUCT NAME	
5-hmC, 5-mC & cytosine DNA standard pack	
Cat. No.: <b>AF-101-0002</b>	<b>Format:</b> 3 tubes each containing 2 µg of 100% unmodified cytosine, 5-methylcytosine, and 5-hydroxymethylcytosine DNA standard.

**Product description**

The **5-hmC, 5-mC & cytosine DNA standard pack** includes hydroxymethylated, methylated and unmethylated DNA standards. The three provided DNA standards are linear dsDNA of ± 300 bp and have been produced by PCR using the Diagenode’s MethylTaq DNA polymerase (Cat. No. AF-103-0250). The templates used for the PCR were DNA from Arabidopsis thaliana chromosome 1 (BAC F19K16) and chromosome 3 (BAC F24B22). The difference is that each contains either normal cytosines, 5-methylcytosines, or 5-hydroxymethylcytosines. In the latter case, the Diagenode’s hydroxymethyl dCTPs (Cat. No. AF-102-0300) have been used instead of normal cytosine nucleotides.

These methylation standards have been thoroughly tested in dot blot (Figure 2).

- Unmethylated control (300bp): [Chr3: 20074379-20074678]
- Methylated control (300bp): [Chr1: 30084349-30084050]
- Hydroxymethylated control (280 bp): [Chr1: 30003709-30003430]

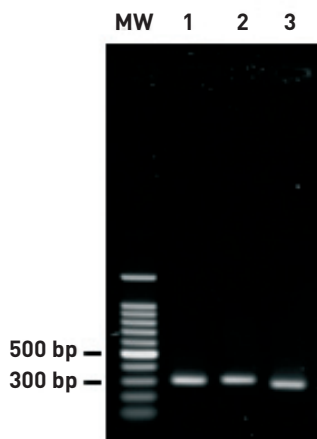
**Storage and stability**

Store at -20°C. Avoid multiple freeze/thaw cycles. For long-term storage, and to prevent cross-contamination. Aliquoting is recommended.

**Applications**

Ideal control DNA for dotblot analysis using the 5-methylcytosine (Cat. No. MAb-081-100/500) and 5-hydroxymethylcytosine (Cat. No. MAb-633HMC-050/100) monoclonal antibodies.

**Quality control**



**Figure 1: Agarose gel electrophoresis**

**MW:** molecular weight marker (100 bp ladders)  
**1:** Unmethylated DNA 300 bp (cytosine)  
**2:** Methylated DNA 300 bp (5-mC)  
**3:** Hydroxymethylated DNA 280 bp (5-hmC)

**Figure 2**  
**5-hmC, 5-mC & cytosine DNA standard pack dot blot analysis**

200 ng of each PCR product (~10 pmol equivalent of C-bases) were spotted on the membrane

1= PCR product containing only 5-hmC bases

2= PCR product containing only 5-mC bases

3= PCR product containing only unmodified C bases

The membrane was incubated with 4 µg/ml 5-hydroxymethylcytosine monoclonal antibody (Cat. No. MAb-633HMC-050, MAb-633HMC-100) (dilution 1:500). A detailed dotblot protocol can be downloaded from our website.



Incubation of the same membrane with the 5-methylcytosine monoclonal antibody (Cat. No. MAb-335-MEC-100/500) (dilution 1:250).



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.