

PRODUCT NAME	
5-hmC, 5-mC & cytosine DNA standard pack for hMeDIP	
Cat. No.: AF-107-0040	Format: 40 rxns

Product content

Description	Comments	Format
5- hmC DNA standard	Hydroxymethylated standard	60 µl
5-mC DNA standard	Methylated standard	60 µl
Cytosine DNA standard	Unmethylated standard	60 µl
5-hmC DNA standard primer pairs	Concentration: 5 µM	100 µl
5-mC standard primer pairs	Concentration: 5 µM	100 µl
Cytosine standard primer pairs	Concentration: 5 µM	100 µl

Product description

The 5-hmC, 5-mC & cytosine DNA standard pack includes 3 hydroxymethylated, methylated and unmethylated DNA standards and their corresponding PCR primer pairs.

The hydroxymethylated DNA standard is a linear dsDNA that has been produced by PCR using the Diagenode’s MethylTaq DNA polymerase [Cat. No. AF-103-0250] and Diagenode’s hydroxymethyl dCTPs [Cat. No. AF-102-0300] replacing normal cytosine nucleotides. The methylated and unmethylated standards have been produced using synthetic oligonucleotides. For the methylated standard, the normal cytosine nucleotides have been replaced by methylcytosine nucleotides. None of these standard show significant homology with human, mouse or rat genomic sequences.

The size of the amplicons produced with the provided primer pairs are:

- Unmethylated control: 69 bp
- Methylated control: 65 bp
- Hydroxymethylated control: 88 bp

Cycling conditions :

Use 1 µl of each DNA standard per qPCR reaction.

qPCR mix (total volume of 25 µl/reaction):

- 6.50 µl of water
- 12.50 µl of master mix (e.g.: iQ SYBR® Green supermix)
- 1.00 µl of provided primer pair (stock: 5 µM each: reverse and forward)
- 5.00 µl of isolated DNA or INPUT

	Temperature	Time	Cycle
PCR Amplification	95°C	7 minutes	x1
	95°C	15 seconds	x40
	60°C	60 seconds	
	95°C	1 minute	x1
Melting Curve	65°C and increment of 0.5°C per cycle	1 minute	x60

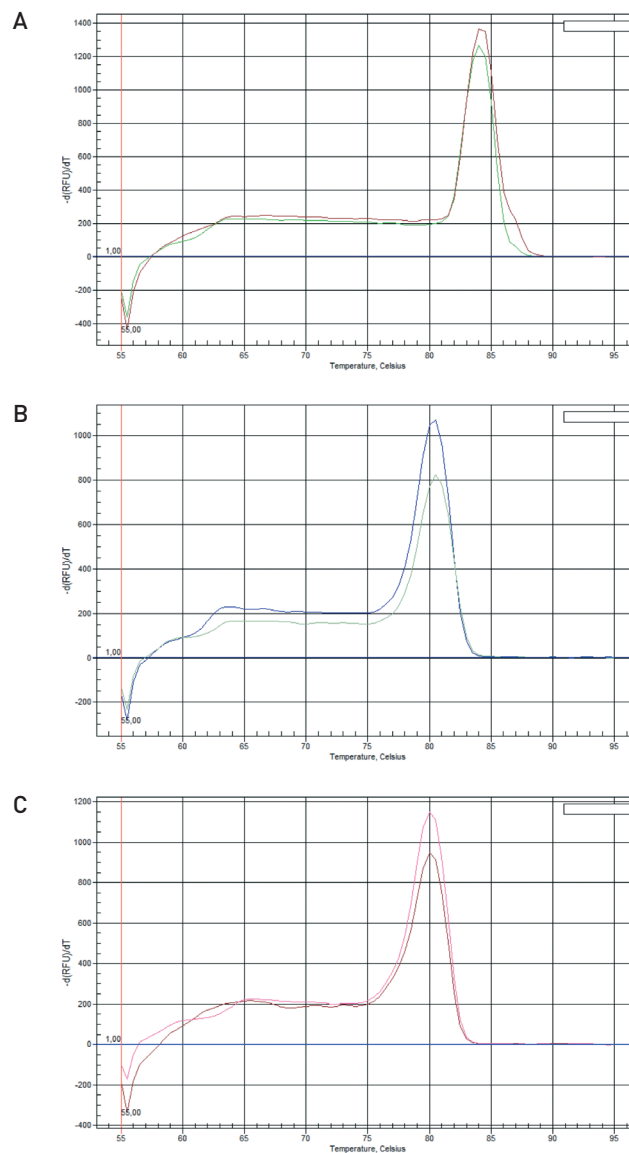
Storage: Store at -20°C. Avoid multiple freeze/thaw cycles. For long-term storage, and to prevent cross-contamination. Aliquoting is recommended.

Applications

Ideal control DNA for hMeDIP analysis using the 5-hydroxymethylcytosine [Cat. No. MAb-633HMC-050/100] monoclonal antibodies included in the hMeDIP [Cat. No. AF-104-0016].

Quality control

Figure 1



Melting curve of PCR product amplified with primer pair for hydroxymethylated DNA (A), methylated DNA (B) and unmethylated DNA (C). See cycling conditions for more details.