Methylated DNA Standard Kit

Catalog No: 55008

Format: 3 x 2.5 µg

Quality Control:

Contents:

3 x 2.5 µg each DNA amplified from the APC gene promoter (50 ng/µl)
1 x 400 µl (2.5 µM) APC PCR Primer mix

A 338 base pair fragment from the APC (adenomatous polyposis coli) gene promoter was amplified by PCR. The resulting dsDNA standard contains 122 cytosine residues on the forward strand, of which 29 occur in a CpG sequence context. The unmethylated, 5-methylcytosine and 5-hydroxymethylcytosine containing versions were created by the inclusion of either unmethylated dCTP, 5-methyl dCTP or 5-hydroxymethyl dCTP in the PCR reaction mix to ensure that all cytosines contain the same methylation state.

The size of the DNA standards are confirmed to contain a single 338 base pair sized band for each standard on a 2.5% agarose gel (see Figure 1).

The identity of the methylated standards was confirmed by dot blot antibody detection as shown in Figure 2 for both ssDNA (lane 1) and dsDNA (lane 2).

The included APC PCR primer mix contains both forward and reverse strand primers to amplify a 195 bp PCR product of the DNA standard (see Figure 3). The APC PCR primers can be used for either endpoint or real time PCR.

For **real time PCR** we recommend the following conditions:

SYBR Green master mix	5 µl
2.5 µM APC PCR primer mix	1.4 µl
Ultrapure sterile water	0.6 µl
DNA template	3 µl
Total	**10 µl**

95°C for 2 min
(95°C for 3 sec; 60°C for 30 sec) for 40 cycles

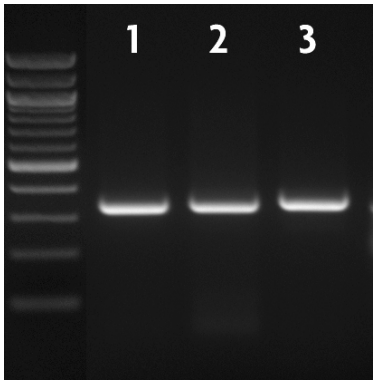

Figure 1:
500 ng of each amplified dsDNA standard was loaded on a 2.5% agarose gel.
Lane 1: unmethylated standard
Lane 2: 5-methylcytosine standard
Lane 3: 5-hydroxymethylcytosine standard

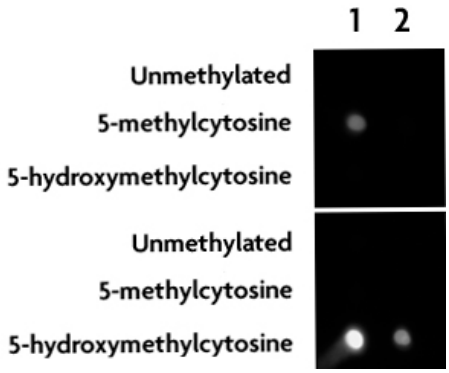

Figure 2: 50 ng of each methylated DNA standard was spotted onto a positively charged nylon membrane. Top panel: 5-Methylcytosine antibody (Catalog No. 39649, 1:1,000 dilution). Bottom panel: 5-Hydroxymethylcytosine antibody (Catalog No. 39769, 1:5,000 dilution).

Quality Control:

For **endpoint PCR** we recommend the following conditions:

Ultrapure sterile water	2.1 µl
5X Phusion GC buffer	4 µl
5 M Betaine	4 µl
100% DMSO	0.6 µl
dNTP mixture (5 mM each)	3.2 µl
2.5 µM APC PCR primer mix	4 µl
Phusion polymerase 2 U/µl	0.1 µl
DNA template	2 µl
Total	**20 µl**

98°C for 1 min
(98°C for 30 sec; 55°C for 30 sec; 72°C for 30 sec) for 32 cycles
72°C for 1 min
Hold at 4°C

Storage and Guarantee: Store at -20°C. This product is guaranteed stable for 6 months from date of receipt when stored properly.

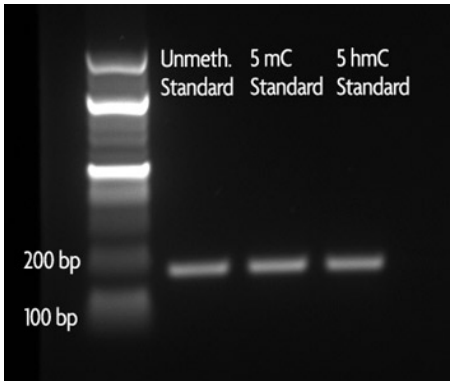

Figure 3:
Endpoint PCR analysis of the Methylated DNA standards using the included APC PCR primer mix shown on a 2% agarose gel.