

## Certificate of Analysis

### Methylated Human Control

**Cat. #**  
N1231

**Size**  
5µg

**Description:** Methylated Human Control<sup>(a)</sup> DNA was purified from a human male source. CpG sites in the DNA were enzymatically methylated by M.SssI methyltransferase to provide a high percentage of methylated CpG motifs.

**Storage Conditions:** Store at 2–10°C.

**Usage Note:** Methylated Human Control DNA can be bisulfite-converted with MethylEdge™ Bisulfite Conversion System (Cat.# N1301) in parallel with experimental samples to assess conversion efficiency.

**Expiration Date:** See product label for expiration date.

**Concentration:** See the product label for lot-specific information.

Part# 9PIN123

Revised 9/16



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### Quality Control Assays

This lot passes the following quality control specifications:

**Percent Methylation:** ≥ 95% methylation of CpG sites as determined by DNA sequencing.



**Promega**

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Signed by:

R. Wheeler, Quality Assurance

<sup>(a)</sup>Use of Methylation Specific PCR (MSP) is protected by U.S. Pat. Nos. 5,786,146; 6,017,704; 6,200,756 and 6,265,171. No license under these patents to use the MSP process is conveyed expressly or by implication to the purchaser by the purchase of this product.

## 1. Purpose

When studying DNA methylation using bisulfite conversion, it is essential that control reactions are run at every step in the process because the presence of a cytosine following bisulfite-conversion indicates methylation. Control DNA should be bisulfite-converted in parallel with experimental samples to ensure that >99% of cytosines are converted and >99% of methylated CpGs are protected. Impurities carried over during purification of source DNA or the presence of secondary structure can affect the efficiency of conversion. Bisulfite-converted control DNA also should be run in parallel with experimental samples in downstream analysis to prevent false-positive identification of methylated cytosines.

## 2. Bisulfite Conversion

Methylated Human Control DNA<sup>(a)</sup> (Cat.# N1231) can be bisulfite-converted using systems such as the MethylEdge™ Bisulfite Conversion System. Although 50pg–1µg can be converted in a single reaction, we recommend using 1–2µl of Methylated Human Control DNA. The concentration following bisulfite conversion can be estimated using a UV absorbance scan and viewing the spectra from 220–350nm using absorbance at 260nm. **Note:** If you are using a NanoDrop® Spectrophotometer, set the Sample Type to “RNA-40” because the converted sample contains uracil and is largely single-stranded.

## 3. Amplification of Bisulfite-Converted DNA

We recommend using either GoTaq® Hot Start Green Master Mix or GoTaq® qPCR Master Mix. Thaw the Master Mix and gently vortex. Both of these master mixes are Hot Start, so the reaction mixes can be set up at room temperature. We recommend amplifying 1–2µl of Converted Methylated Human Control DNA<sup>(a)</sup> (Cat.# N1221) per reaction in parallel with experimental samples.

### Suggested Reaction Mix

| Component           | Volume                        | Final Concentration |
|---------------------|-------------------------------|---------------------|
| 2X PCR Master Mix   | 12.5µl                        | 1X                  |
| upstream primer     | Xµl                           | 0.2–0.9µM           |
| downstream primer   | Xµl                           | 0.2–0.9µM           |
| DNA template        | 1–2µl                         | 20–50ng             |
| Nuclease-Free Water | Xµl to a final volume of 25µl |                     |

**Note:** Although typically not necessary, optimizing the magnesium concentration might improve the yield for some targets. When supplementing with magnesium, adjust the Nuclease-Free Water volume to maintain a final volume of 25µl.

**Table 1. Cycling Conditions for Endpoint PCR.**

| Step              | Temperature | Time          | Number of Cycles |
|-------------------|-------------|---------------|------------------|
| Enzyme Activation | 95°C        | 2 minutes     | 1                |
| Denaturation      | 95°C        | 15 seconds    | 40               |
| Annealing         | Variable    | 30–60 seconds |                  |
| Extension         | 72°C        | 60 seconds    |                  |
| Final Extension   | 72°C        | 5 minutes     | 1                |

**Table 2. Cycling Conditions for Real-Time PCR.**

| Step              | Temperature | Time          | Number of Cycles |
|-------------------|-------------|---------------|------------------|
| Enzyme Activation | 95°C        | 2 minutes     | 1                |
| Denaturation      | 95°C        | 15 seconds    | 40               |
| Annealing         | Variable    | 30–60 seconds |                  |
| Dissociation*     | 65–95°C     | variable      | 1                |

\*Optional

## 4. Recommendations for PCR Primer Designs

### General Considerations

Primer design is key to analyzing bisulfite-converted DNA using PCR-based methods. Primers must be carefully designed based on the converted sequence to avoid PCR bias. Keep in mind that following conversion, DNA strands are no longer complementary and, because the DNA sequence is now reduced to essentially three bases (A, U, G), there is higher probability for non-specific interaction. Unconverted DNA should be run in parallel with bisulfite-converted DNA to ensure the primers are specific to the bisulfite-converted sequence. Several tools are available online to assist in developing primers specific to bisulfite-converted DNA, such as MethPrimer ([www.urogene.org/methprimer/index1.html](http://www.urogene.org/methprimer/index1.html)).

Primers for experimental DNA samples of poor quality (e.g., DNA isolated from FFPE tissue) should be designed to yield amplicons smaller than 200bp.

### Real-Time PCR Considerations

Because bisulfite conversion results in highly fragmented DNA, smaller amplicons will yield better results. Amplicons for real-time PCR should be 75–200bp. If larger amplicons are required, be sure to optimize reaction conditions using control DNA to verify efficiency.

### End-Point PCR Considerations

The MethylEdge™ Bisulfite Conversion System (Cat.# N1301) yields bisulfite-converted DNA with significantly less fragmentation than other bisulfite conversion kits. When using this system, amplicons for end-point PCR can be designed up to 500bp when high-quality, purified genomic DNA is used. Amplicons larger than 700bp have been successfully amplified with highly optimized primer models. Amplification of longer sequences may require more template DNA and/or higher primer concentration.

## 5. Related Products

| Product                                 | Size            | Cat.# |
|---|-----------------|-------|
| MethylEdge™ Bisulfite Conversion System | 50 reactions    | N1301 |
| Converted Methylated Human Control      | 1µg             | N1221 |
| ReliaPrep™ FFPE gDNA Miniprep System    | 10 reactions    | A2351 |
|   | 100 reactions   | A2352 |
| ReliaPrep™ Blood gDNA Miniprep System   | 100 preps       | A5081 |
|   | 250 preps       | A5082 |
| ReliaPrep™ gDNA Tissue Miniprep System  | 100 preps       | A2051 |
|   | 250 preps       | A2052 |
| GoTaq® Hot Start Green Master Mix       | 10 reactions    | M5121 |
|   | 100 reactions   | M5122 |
|   | 1,000 reactions | M5123 |
| GoTaq® qPCR Master Mix                  | 200 reactions   | A6001 |
|   | 1,000 reactions | A6002 |
| QuantiFluor® dsDNA System               | 1ml             | E2670 |
| QuantiFluor® ssDNA System               | 1ml             | E3190 |

<sup>(a)</sup>Use of Methylation Specific PCR (MSP) is protected by U.S. Pat. Nos. 5,786,146; 6,017,704; 6,200,756 and 6,265,171. No license under these patents to use the MSP process is conveyed expressly or by implication to the purchaser by the purchase of this product.