

## User's Manual and Instructions

# Methylated Human DNA Standard and Control Primers

**Product Name: Methylated Human Genomic DNA Control**  
**Cat #: D6255815**

**Storage Condition: -20°C**

**Product Contents:**

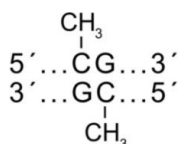
Cat. # <b>D6255815</b>	
Methylated Human Genomic DNA Control	5 µg/20 µl
Control Primer mixture	10 ul

**Shipping Condition: Dry Ice**

**Shelf Life: One year from the date of receipt under proper storage condition**

**Description**

The methylated human genomic DNA was generated by treating Jurkat cell genomic DNA with Methylase, M. SssI, which specifically methylates the CpG into C<sup>m</sup>pG within double stranded dinucleotide recognition sequence. The Fully methylated Jurkat genomic DNA is provided undigested and can be used as methylated control DNA for research.



The primer set is designed to amplify a human gene fragment following bisulfite treatment. The methylated CG remains unconverted following bisulfite treatment, whereas non-methylated cytosines are converted into uracil and detected as thymine after PCR. The 220bp PCR product can be detected on a 3% DNA gel.

**PCR condition:**

**Notice:** Before start PCR the **Methylated Human Genomic DNA Control** has to be converted with bisulfite (xxxxxxx kit)

A. PCR Setup:

For a 25 µl total reaction volume:

1. add 5µl Converted methylated control DNA

2. add 1µl primer mixture

Standard PCR buffer with 10 mM dNTP mix

MgCl<sub>2</sub> 2.5 mM, if needed

DNA Polymerase

Add water to 25 µl

B. PCR condition:

1. 95 °C, 5 minutes
2. 95 °C, 40 seconds
3. 60 °C, 40 seconds
4. 72 °C, 40 seconds
5. Repeat steps 2 through 4 for 45 times.
6. 72 °C, 4 minutes
7. 4 °C

The PCR production is also can be confirmed by sequencing: Only methylated C in the CG sequences remains as C after conversion. This means that C is converted to T except for the methylated CG. Thus CC, CT or CA sequences will not be detected.

TTTTCGCGTTAGAGACCGTAGTCCGCGTTTTTATTATTTATATTTATCGCGTTTTTCGTTCGTTTTTTTTTTCG  
GGAGTTAGTTCGCGTTATCGTCGTTGTTTAGGTTATCGTTATTTTTCGTAGTTATGTTTATTAGGTTCG  
TGTTTTCGTTTTTTTTATCGTAGGATGTTCGGCGGTTCGGGTTATCGCGAGTCGGTTCGAGTTTT

#### Quality Control

1. The methylated genomic DNA is tested with the control primer, a xxx size band can visualized on 1% agarose gel.
2. The C<sup>m</sup>pG sequences are confirmed by sequencing analysis after bisulfite conversion.
3. The methylated human genomic DNA purity is tested by spectrophotometer, A260/280 is between 1.7 and 2.0, A260/230 is >2.0. (detected in 10 mM Tris-Cl, pH 7.5)

#### Components

1. Methylated Human Genomic DNA 5 µg, at concentration 250 ng/ul
2. Control Primer Pair
3. Protocol Manual
4. Certificate of Analysis
5. MSDS