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User's Manual and Instructions

Universal Methylated Human DNA Standard and Control Primers

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

Storage Condition: -20ºC

Product Contents:

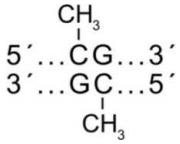
Cat. # D **D6255815**	
Methylated Human Genomic DNA Control	5 µg/20 µl
Control Primer mixture	10 ul

Shipping Condition: Blue 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 $C^{m}pG$ within double stranded dinucleotide recongnition 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 (xxxxxxxx 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
MgCl2 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 conformed by sequencing: Only CG sequences are stay in CG without converted and no CC, CT or CA sequences can be detected. That means the C is converted to T except the methylated CG.

TTTTCGCGTTAGAGACGTAGTCGCGTTTTTATTATTTATATTTATCGCGTTTTCGTTCGTTTTTTTTTCG
GGAGTTAGTTCGCGTTATCGTCGTTGTTTAGGTTATCGTTATTTTTCGTAGTTATGTTTATTAGGTTCG
TGTTTTCGTTTTTTTTATCGTAGGATGTTCGGCGGTTCGGGTATCGCGAGTCGGTCGAGTTTT

Quality Control

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^{m}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