Tel: 1-888-762-2568 Fax: 1-510-783-5386 Email: info@biochain.com

# User's Manual and Instructions

**Methylated/Low-Methylated Human DNA Standard Pairs and Control Primers**

**Product Name: Methylated & Low-Methylated DNA Matched Pairs**

**Cat #: D6234148-PP, D6234149-PP, and D6254874-PP**
**Storage Condition:** -20ºC

**Product Contents:**

| Cat. # **D6234148-PP, D6234149-PP, and D6254874-PP** | |
|---|---|
| Methylated Human Genomic DNA Control | 5 µg/20 µl (250 ng/ul concentration) |
| Low-Methylated Human Genomic DNA Control | 5 µg/20 µl (250 ng/ul concentration) |
| Control Primer mixture L | 10 ul |
| Control Primer mixture M | 10 ul |

**Shipping Condition:** Dry Ice

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

**Description:**

Our methylated and low-methylated matched pair DNA can be utilized as positive and negative controls, respectively, in research focusing on gene methylation studies.

The low-methylated genomic DNAs are selected from human young donor blood peripheral leukocyte, liver tissue, or lymphocyte cell line. The DNAs derived from these sources naturally have a low level of methylation and display batch-to-batch consistency. Unlike DNAs from the DNA methyltransferase genetic knockout cell lines, these DNA sources are ideal for more natural control DNAs to be used in gene methylation studies.

The methylated human genomic DNAs are generated by treating the same low-methylated DNA with Methylase, M. SssI, which specifically methylates the CpG into $C^{m}pG$ within double-stranded dinucleotide recongnition sequence (Fig. 1). Both low-methylated and fully methylated genomic DNA are provided undigested.

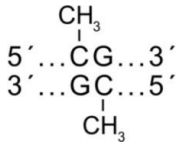


**Figure 1.** M.SssI methylates all cytosine residues within double-stranded CpG sequence motifs.

The primer sets are designed to amplify a specific and methylated region of the human vimentin gene following bisulfite treatment. The methylated CG remains unconverted following bisulfite treatment, whereas low-methylated cytosines are converted into uracil and detected as thymine after PCR. Following amplification, a 220bp PCR product can be detected on a 3% DNA gel for the fully methylated DNA, whereas the low-methylated DNA will not produce any bands when using the M primer set.

**PCR condition:**

**Notice**: *Before starting PCR the* **Methylated & Low-methylated DNA Matched Pair** *has to be converted with bisulfite (Cat. No. K5082100).*

A. PCR Setup:

For a 25 µl total reaction volume:

- add 5 µl *Converted methylated or converted low-methylated control DNA*
- add 1 µl primer mixture
- add standard PCR buffer with 10 mM dNTP mix
- add $MgCl_2$ 2.5 mM, if needed
- add DNA Polymerase
- add water to 25 µl

B. Recommended PCR condition:

- 95 °C, 5 minutes
- 45 cycles of 95 °C, 40 seconds, 60 °C, 40 seconds, and 72 °C, 40 seconds
- 72 °C, 4 minutes then hold at 4 °C

The PCR product can also be confirmed by sequencing: Only methylated C in the CG sequences remain 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.

**Validation:**

Our genomic DNA is confirmed by PCR with the provided primer sets following bisulfite treatment. The M primer set can only generate PCR product for the methylated DNA standard, and the L primer set can only generate PCR product for the low-methylated DNA standard. The PCR product can be further analyzed by sequencing.

The following shows the expected sequence of interest from the original vimentin gene:

ACCACCCACACCCAC**CGCG**CCCT**CG**TT**CG**CCTCTTCTC**CG**GGAGCCAGTC**CGCG**CCAC**CG**C**CG**C**CG**CCCAGGCCAT**C**
**G**CCACCCTC**CG**CAGCCATGTCCACCAGGTC**CG**TGTCCT**CG**TCCTCCTAC**CG**CAGGATGTT**CG**G**CG**GCC**CG**GGCAC

*Human Low-methylated DNA*[1]: Below is the expected sequence for the Human Low-methylated DNA after bisulfite conversion and PCR with L primer set (sense strand). During treatment with sodium bisulfite, low-methylated cytosines are converted into uracils, which are later detected as thymines after PCR.

ATTATTTATATTTAT**TGTG**TTTT**TG**TT**TG**TTTTTTTTT**TG**GGAGTTAGTT**TGTG**TTAT**TG**T**TG**T**TG**TTTAGGTTAT**T**
**G**TTATTTTT**TG**TAGTTATGTTTATTAGGTT**TG**TGTTTT**TG**TTTTTTTAT**TG**TAGGATGTT**TG**G**TG**GTT**TG**GGTAT

*Human Methylated DNA*[2]: Below is the expected sequence for the Human Methylated DNA after bisulfite conversion and PCR with M primer set (sense strand). Methylated cytosines in the CpG dinucleotide context remain unconverted following bisulfite treatment, whereas non-methylated cytosines, or cytosines not in the CpG context, are converted to uracils and detected as thymines after PCR.

ATTATTTATATTTAT**CGCG**TTTT**CG**TT**CG**TTTTTTTTT**CG**GGAGTTAGTT**CGCG**TTAT**CG**T**CG**T**CG**TTTAGGTTAT**C**
**G**TTATTTTT**CG**TAGTTATGTTTATTAGGTT**CG**TGTTTT**CG**TTTTTTTAT**CG**TAGGATGTT**CG**G**CG**GTT**CG**GGTAT

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[1]The sequencing results may show small cytosine peaks superimposed on the thymine peaks within the context of a TG motif. This result is normal, as it may be difficult to completely bisulfite-convert certain sequences. However, these signals do not affect the sequence result.

[2]The sequencing results may show small thymine peaks superimposed on the cytosine peaks within the context of a CG motif. This results from the known observation that during PCR, any non-methylated template remaining after the methyltransferase reaction is more efficiently amplified than the methylated template. When considering this bias and that it does not affect the sequence result, the genomic DNA can be considered to be virtually completely methylated.