

# Mouse Premixed Methylation Calibration Standards



## Product Highlights

- ▶ Premixed levels of Mouse High and Low methylation control DNA can be used to quantify the methylation levels of unknown samples or to validate quantitative methylation assays
- ▶ Ideal for use as controls in bisulfite methylation analysis procedures including Pyrosequencing, NGS, and MS-HRM

## Product Contents

- ▶ 1 vial 0% Mouse Methylated Genomic DNA (1 µg at 50 ng/µL)
- ▶ 1 vial 10% Mouse Methylated Genomic DNA (1 µg at 50 ng/µL)
- ▶ 1 vial 25% Mouse Methylated Genomic DNA (1 µg at 50 ng/µL)
- ▶ 1 vial 50% Mouse Methylated Genomic DNA (1 µg at 50 ng/µL)
- ▶ 1 vial 75% Mouse Methylated Genomic DNA (1 µg at 50 ng/µL)
- ▶ 1 vial 90% Mouse Methylated Genomic DNA (1 µg at 50 ng/µL)
- ▶ 1 vial 100% Mouse Methylated Genomic DNA (1 µg at 50 ng/µL)

## Ordering Information

| CATALOG NUMBER  | PRODUCT   | PRICE    |
|-----------------|---|----------|
| 80-8060M-PREMIX | Mouse Premixed Calibration Standard (1 µg per vial, 20 µL volume) | \$402.40 |

## Related Products

| CATALOG NUMBER   | PRODUCT  | PRICE     |
|------------------|--|-----------|
| 80-8061-HGHM5    | Human high methylated genomic DNA (5 µg at 100 ng/ µL)                                       | \$242.83  |
| 80-8063-MGHM5    | Mouse high methylated genomic DNA (5 µg at 100 ng/ µL)                                       | \$252.56  |
| 80-8065-RGHM5    | Rat high methylated genomic DNA (5 µg at 100 ng/ µL)   | \$252.56  |
| 80-8067-PMGHM5   | Monkey ( <i>Macaca mulatta</i> ) high methylated genomic DNA (5 µg at 100 ng/ µL)            | \$281.63  |
| 80-8062-HGUM5    | Human low methylated genomic DNA (5 µg at 100 ng/ µL)  | \$242.83  |
| 80-8064-MGUM5    | Mouse low methylated genomic DNA (5 µg at 100 ng/ µL)  | \$2452.56 |
| 80-8066-RGUM5    | Rat low methylated genomic DNA (5 µg at 100 ng/ µL)  | \$252.56  |
| 80-8068-PMGUM5   | Monkey ( <i>Macaca mulatta</i> ) low methylated genomic DNA (5 µg at 100 ng/ µL)             | \$281.63  |
| 80-8060H-PREMIX  | Human Premixed Calibration Standard (1 µg per vial, 20 µL volume)                            | \$386.56  |
| 80-8060R-PREMIX  | Rat Premixed Calibration Standard (1 µg per vial, 20 µL volume)                              | \$402.40  |
| 80-8060PM-PREMIX | Monkey ( <i>Macaca mulatta</i> ) Premixed Calibration Standard (1 µg per vial, 20 µL volume) | \$412.46  |

## Technical Specifications

- ▶ 5µg DNA in TE buffer (10mM Tris-HCl, 1mM EDTA, pH 8.0)
- ▶ Store at -20°C, in aliquots, for 2 years. For best results, do not freeze/thaw an individual aliquot more than three times. For longer term storage -70°C is recommended.
- ▶ Requires bisulfite conversion prior to use.

## Example Protocol

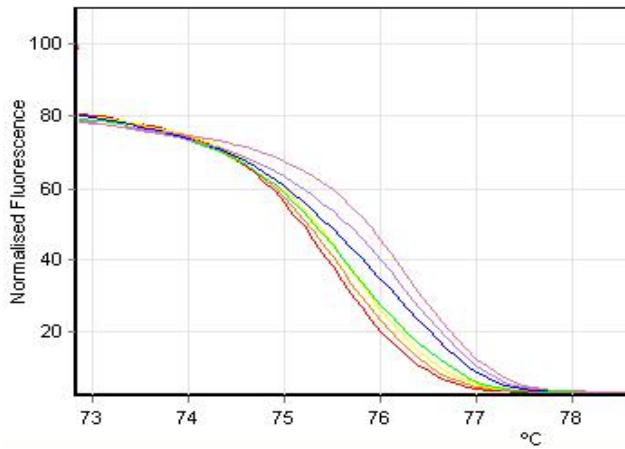
- ▶ Bisulfite modification of Premix and sample(s) of interest.
  - Zymo Research EZ Methylation kit (Cat.#D5002 or D5004) with 200 - 500 ng of input DNA following manufacturers recommended protocol.
- ▶ PCR amplification Protocol

| Component   | Per 30µl reaction             |
|---|-------------------------------|
| 10X PCR buffer (Contains 15mM MgCl <sub>2</sub> ) | 3 µl (1x)                     |
| 25 mM MgCl <sub>2</sub>                           | 1.8 µl (3.0 mM final conc.)   |
| 10 mM dNTPs                                       | 0.6 µl (200 µM of each)       |
| 10 µM Fwd primer                                  | 0.6 µl (6 pmol)               |
| 10 µM Rev primer                                  | 0.6 µl (6 pmol)               |
| HotStar Taq Polymerase (5 U/µl)                   | 0.15 µl (0.75 U)              |
| DNA   | 1 µl of bisulfite treated DNA |
| Water   | Adjust to 30 µl               |

- HotStar Taq Polymerase Qiagen Cat. #203205 recommended with the following PCR cycling conditions:
  - 95°C 15 min; 45 x (95°C 30s; Ta°C 30 s; 72°C 30 s); 72°C 5 min; 4°C ∞
- Additional optimization is needed if different PCR system is used in analysis.
- ▶ Sequencing Analysis: Pyrosequencing, NGS, or MS-HRM.

## Example Quality Control Results

Figure: High Resolution Melt (HRM) Analysis Results



loses its fluorescence.

*(Right) Example HRM Standard Curve. The fluorescence at a certain temperature can be used to differentiate between different levels of methylation. Lower levels of methylation correspond to greater numbers of cytosine to uracil conversions due to bisulfite treatment and, consequently, lower melting temperatures.*

Premixed Control DNA is used to calibrate the HRM analysis assay, which measures the percent methylation of a bisulfite-converted DNA amplicon. **(Left)** Example HRM Melting Curve. The amount of DNA that has melted into single-stranded molecules is measured by the fluorescence of the sample after it is mixed with a DNA-intercalating dye. When a double-stranded DNA molecule melts into single-stranded DNA molecules, the dye

