

# Human Premixed Methylation Calibration Standards



## Product Highlights

- ▶ Premixed levels of Human 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% (Low Methylated) Human Methylated Genomic DNA (1 µg at 50 ng/µL)
- ▶ 1 vial 10% Human Methylated Genomic DNA (1 µg at 50 ng/µL)
- ▶ 1 vial 25% Human Methylated Genomic DNA (1 µg at 50 ng/µL)
- ▶ 1 vial 50% Human Methylated Genomic DNA (1 µg at 50 ng/µL)
- ▶ 1 vial 75% Human Methylated Genomic DNA (1 µg at 50 ng/µL)
- ▶ 1 vial 90% Human Methylated Genomic DNA (1 µg at 50 ng/µL)
- ▶ 1 vial 100% (High Methylated) Human Methylated Genomic DNA (1 µg at 50 ng/µL)

## Ordering Information

CATALOG NUMBER	PRODUCT	PRICE
80-8060H-PREMIX	Human Premixed Calibration Standard (1 µg per vial, 20 µL volume)	\$386.56

## 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)	\$252.56
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
80-8060M-PREMIX	Mouse Premixed Calibration Standard (1 µg per vial, 20 µL volume)	\$402.40
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

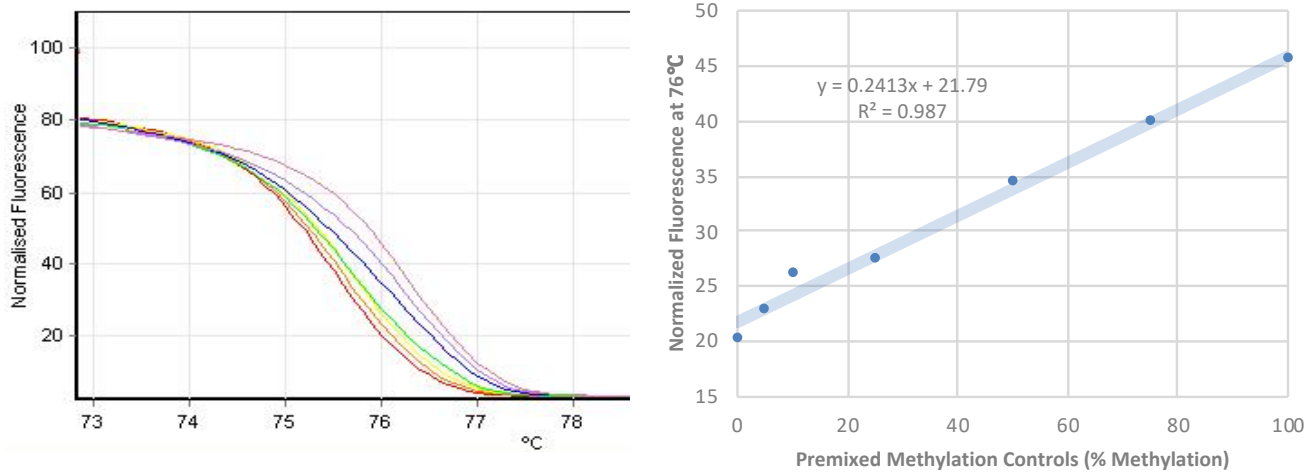
- ▶ Bisulfite modification of Premix and sample(s) of interest.
  - Zymo Research EZ Methylation kit (Cat.#D5002 or D5004) with 200 - 500 ng of input DNA following manufacturers recommended protocol.
- ▶ PCR amplification Protocol

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



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 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.