Human Premixed Methylation Calibration Standards

Product Name: Human PreMix Methylated DNA Control

Intended Use: FOR RESEARCH USE ONLY

Ordering Information

CATALOG NUMBER	PRODUCT	PRICE
80-8060H-PREMIX	Human Pre-mixed methylation controls at: Low (0%), 10%, 25%, 50%, 75%, 90%, and high (100%)	$405.50

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)

Technical Specifications: 1µg each in TE buffer (10mM Tris-HCl, 1mM EDTA, pH 8.0) Store at -20°C, upon arrival, 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.

Shipping: Dry ice, Ice pack, or ambient (20°C) Product Highlights

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

Contact our office by email or by phone to place an order

EpigenDx, Inc
96 South Street, Hopkinton, MA 01740
Email: info@epigendx.com
Tel: (508)-497-9400
Toll Free: (800)-941-6884
Fax: (508)-497-9450
Website: Labs.epigendx.com

Technical Specifications

- 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 $MgCl_2$)	3 µL (1x)
25 mM $MgCl_2$	1.8 µL (3.0 mM final conc.)
10 mM dNTPs	0.6 µL (200 µM of each)
10 µM Forward primer	0.6 µL (6 pmol)
10 µM Reverse primer	0.6 µL (6 pmol)
HotStart Taq Polymerase (5 U/µl)	0.15 µL (0.75 U)
DNA	1 µL of bisulfite treated DNA
Water	Adjust to 30 µL

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

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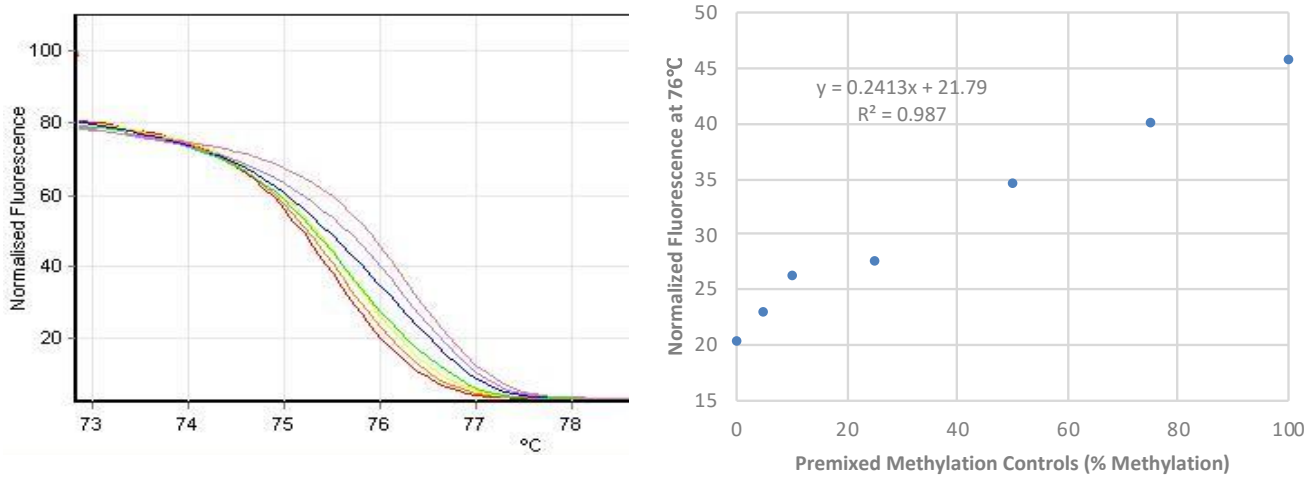
Example Quality Control Results

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.*

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