METHYLDETECT
DNA METHYLATION ASSAYS

Technical Guide: How Does MS-HRM Work?

Introduction

Methylation-Sensitive High-Resolution Melting (MS-HRM) is a PCR-based methylation detection method that enables cost-effective, fast, and highly sensitive assessment of locus-specific DNA methylation status.

Mechanism & Thermal Stability

The assay relies on sequence changes introduced during sodium bisulfite modification to differentiate methylated from unmethylated loci:

- **Cytosine Preservation vs. Conversion:** Sodium bisulfite preserves methylated cytosines while converting non-methylated cytosines into uracil, which are subsequently substituted with thymine during PCR amplification.
- **C-rich vs. T-rich Amplicons:** Amplification of a methylated locus yields a C-rich PCR product, whereas a non-methylated locus yields a T-rich PCR product.
- **Melting Profiles:** Due to higher GC-content, methylated PCR products melt at a higher temperature compared to T-rich non-methylated products, which melt earlier in the temperature gradient.

Sequence Comparison

- **A: Methylated locus PCR product (C-rich):**

```
5′— TtcGTTAAttcGGcGcGTTTcGcGATtttTGG —3′
3′— AagCAATTaagCCCgCgCAAAgCgCTAaaaACC —5′
```

- **B: Non-methylated locus PCR product (T-rich):**

```
5′— TttGTTAAtttGGtGtGTTTtGtGATtttTGG —3′
3′— AaaCAATTaaaCCCaCaCAAAaCaCTAaaaACC —5′
```

Methyl Detect ApS
CVR: DK38322761
Technical Support & Quotes: info@methyldetect.com www.methyldetect.com
Document Notice: Effective as of September 2026. FOR RESEARCH USE ONLY (RUO). Not for use in diagnostic procedures. © 2016-2026 Methyl Detect ApS. All rights reserved.

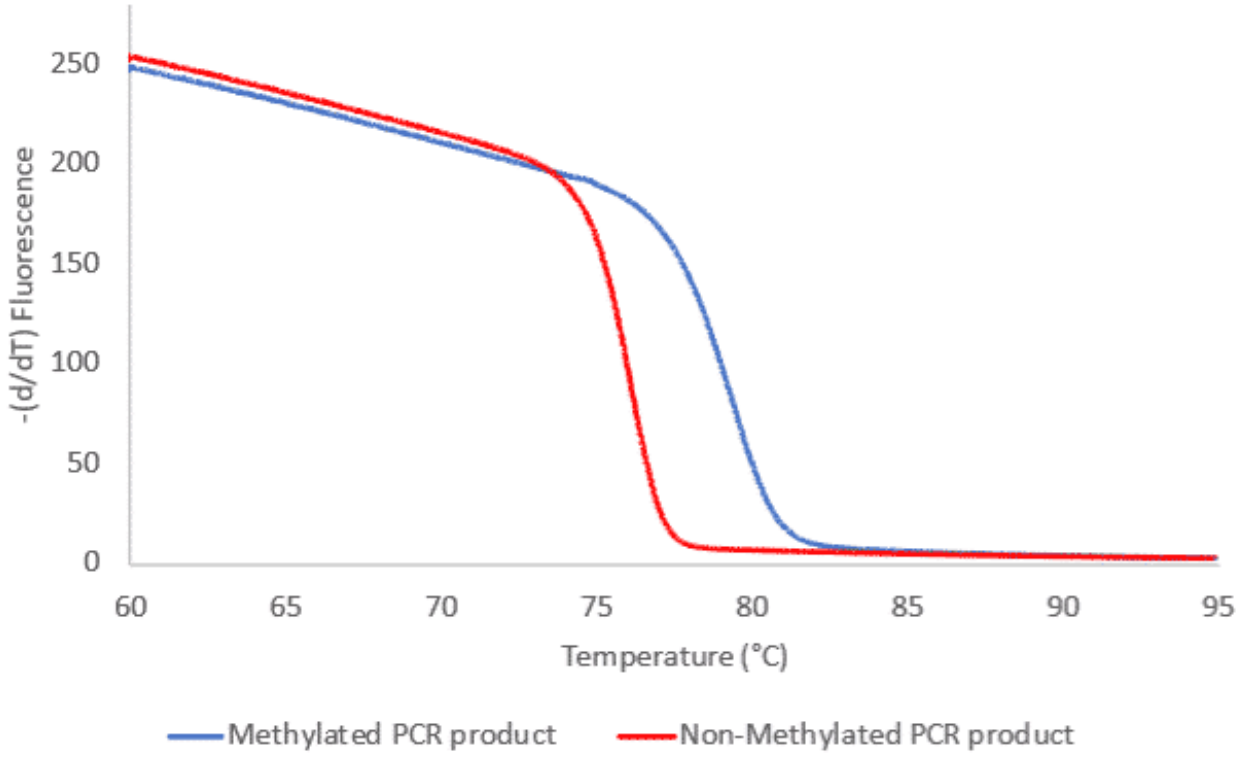

Figure 1: Melting curve plot showing the earlier red peak for non-methylated DNA vs. the higher-temperature blue peak for methylated DNA

Further Reading & Key Literature

- MS-HRM Protocol: *Nature Protocols* (*PMID: 19180074*)
- MS-HRM Validation: *Nucleic Acids Research* (*PMID: 17289753*)
- MS-HRM Applications: *Methods in Molecular Biology (PMID: 18987818, PMID: 29224163)*

Assay Support & Applications

MethylDetect provides pre-validated, ready-to-use EpiMelt MS-HRM assays and master mixes designed for standard qPCR instruments.

For technical assistance or quote requests, contact **info@methyldetect.com** or visit **www.methyldetect.com**.

Methyl Detect ApS
CVR: DK38322761
Technical Support & Quotes: info@methyldetect.com www.methyldetect.com
Document Notice: Effective as of September 2026. FOR RESEARCH USE ONLY (RUO). Not for use in diagnostic procedures. © 2016-2026 Methyl Detect ApS. All rights reserved.