

EpiMelt MS-HRM 2x Master Mix

MADE IN DENMARK



Cat. No.: A553401

A553401

200 reactions (20 µl/reaction)

	EpiMelt MS-HRM 2x Master Mix	ROX internal reference dye, 200 µM
ID No.	5000400	5700300
Colour code	Amber	Amber
Content	2 x 1 ml	1 x 0.05 ml

Key Features

- All-in-one optimized master mix, including HRM dye
- Optimized for MS-HRM analysis
- Assessment of methylation status
- Compatible with *EpiMelt kit for DNA Methylation assessment*

Introduction

EpiMelt MS-HRM 2x Master Mix is an optimized and ready-to-use Master Mix intended for sensitive analysis of the methylation status of CpG-dinucleotides in bisulfite converted DNA. The methylation-sensitive high-resolution melting (MS-HRM) method is based on the comparison of the melting profiles of PCR products from unknown samples with profiles specific for PCR products derived from methylated and non-methylated control DNAs.

The EpiMelt MS-HRM 2x Master Mix, a single-tube 2x reagent, includes MgCl₂, dNTPs, fluorescent DNA dye SYTO 9, and a balanced combination of buffer components formulated specifically for MS-HRM analysis. Just add your primers and your template DNA. The master mix is optimized to be used with the *EpiMelt kit for DNA Methylation assessment*.

The TEMPase Hot Start DNA Polymerase is activated within the EpiMelt MS-HRM 2x Master Mix by a 15 min incubation step at 95°C. This hot start step prevents extension of non-specifically annealed primers and primer-dimers formed at low temperatures during PCR setup.

Composition of EpiMelt MS-HRM 2x Master Mix:

- TEMPase Hot Start DNA Polymerase
- Optimized buffer system including dNTPs, MgCl₂ and the fluorescent DNA dye SYTO 9
- ROX internal reference dye provided in a separate tube

Instrument compatibility: Real-time PCR instruments with the capability to perform melt curve analysis. If internal reference dye is required, please refer to the section "Reference Dye" and "Protocol" for low Rox (30 µM) and high Rox (300 µM). The fluorescent DNA dye SYTO 9 displays the same spectra as SYBR Green and is compatible with all common real-time PCR instruments. Just select the standard settings for FAM.

Recommended Storage and stability

Long term storage at -20 °C. Product expiry at -20 °C is stated on the label.

Option: Store at +4 °C for up to 3 months.

Quality Control

TEMPase DNA Polymerase is tested for contaminating activities, with no traces of endonuclease activity, nicking activity, or exonuclease activity.

The EpiMelt MS-HRM 2x Master Mix is functionally tested using the EpiMelt kit control samples.

Pre-protocol Considerations

Bisulfite Modification

To analyze the methylation status of cytosines within the locus of interest the information about which cytosines are methylated needs to be preserved before PCR amplification is performed. Sodium bisulfite only deaminates non-methylated cytosines to uracil and leaves methylated cytosines untouched. As a result, methylated and unmethylated templates have different sequences which can be distinguished by HRM analysis.

PCR Primers for MS-HRM

Primers for the MS-HRM analysis must be designed to anneal to the converted DNA sequence after bisulfite treatment in such a way that they amplify both methylated and non-methylated DNA templates. The *EpiMelt kit for DNA Methylation assessment* includes primer mix designed for sensitive detection of DNA methylation.

Reference Dye

ROX (6-carboxy-X-rhodamine) internal reference dye is included in this kit and serves as an internal reference for normalization of the fluorescent signal when using real-time PCR instruments, which can detect ROX. ROX corrects well-to-well variations due to pipetting inaccuracies and fluorescence fluctuations. The presence of ROX does not interfere with the qPCR amplification. The excitation and emission of the reference dye are 584 nm and 612 nm, respectively. ROX has direct influence on the ΔR_n amplification plot. Thus, the C_q-value and the amplification plot plateau are influenced by how precisely ROX is added. Therefore, always be meticulous when pipetting.

Preventing Template Cross-Contamination

Due to the high sensitivity of real-time PCR there is a risk of contaminating the reactions with the DNA of previous runs. To minimize this risk, tubes or plates containing reaction products should not be opened or analysed by gel electrophoresis in the same laboratory area used to set up reactions.

Protocol

Follow the protocol in the Handbook for the *EpiMelt kit for DNA Methylation assessment*.

If needed, prepare a fresh dilution of ROX internal reference dye. For a final reaction concentration of 30 nM dilute 1:100 in PCR grade water. For a final reaction concentration of 300 nM dilute 1:10 in PCR grade water. Add 0.3 µl of ROX dilution per 20 µl final reaction volume.

The diluted ROX reference dye must be kept in a light-protected tube at 4 °C.

30 nM ROX is recommended for Applied Biosystems® 7500, 7500 Fast and ViiA™ 7, QuantStudio™ instruments, Agilent Mx3000P™, Mx3005P™, Mx4000™ and AriaMx.

300 nM ROX is recommended for Applied Biosystems® 5700, 7000, 7300, 7700, 7900, 7900 HT, StepOne™ and StepOnePlus™.

Accessories (to be ordered separately)

Reagents	Cat. No.
25mM MgCl ₂ , 3 × 1.5 ml	A308103
ROX™ Internal Reference Dye 200 µM, 3 x 0.2 ml	A351513

Related Products

Real-time PCR Master Mixes (400 x 25 µl reactions)	Cat. No.
RealQ Plus 2x Master Mix for probe, <ul style="list-style-type: none">• without ROX™• with low ROX™• with high ROX™	A313402 A314402 A315402
RealQ Plus 2x Master Mix Green <ul style="list-style-type: none">• without ROX™• with low ROX™• with high ROX™	A323402 A324402 A325402

Reagents for *in vitro* laboratory use only.

Other product sizes, combinations and customized solutions are available. Please look at www.ampliqon.com or ask for our complete product list for PCR Enzymes. For customized solutions please contact us.

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