METHYLDETECT

EpiMelt Real-Time PCR Master Mix

High specificity ready-to-use mix for real-time Hot Start PCR with EvaGreen®.
Optimized for epigenetic analysis using HRM technology. 2× concentrated.

For 200 & 500 reactions in 20 µL

Catalog #	Size
# EPI - qPCR - 200	200 reactions in 20 µL
# EPI - qPCR - 500	500 reactions in 20 µL

Store at -20°C upon arrival

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Contents

EpiMelt Real-Time Master Mix contents

	200 Reactions	500 reactions	Storage
EpiMelt Master Mix 2×	2 x 1 mL	5 x 1 mL	-20°C, in darkness
EpiMelt Sterile Water	2 x 1.5 mL	5 x 1.5 mL	-20°C

Table 1. Contents of the EpiMelt Real-Time PCR Master Mix.

EpiMelt Master Mix 2× composition

Component	Amount
EpiMelt Hot Start DNA Polymerase	0.1 U/µL
$MgCl_2$	4 mM
dNTPs	0.5 mM of each dNTP
2× reaction buffer with EvaGreen®	

Table 2. EpiMelt Master Mix 2× composition.

Additional equipment and reagents

- EpiMelt Bisulfite Modification Standard Kit (MethylDetect)
- Vortex
- Microcentrifuge
- Real-time thermocycler

Important notes

- Before use, all solutions should be thawed thoroughly on ice, gently mixed by inverting, and briefly centrifuged
- Up to 3× repeated freeze-thaw cycles do not influence the activity of this product

Protocol

The EpiMelt Bisulfite Modification Standard Kit (MethylDetect) is recommended for the bisulfite conversion of template DNA.

- Thaw EpiMelt Master Mix 2× and Sterile Water on ice, gently mix by inverting, and briefly centrifuge. Keep on ice.
- Prepare the PCR mix by adding the following components in the order listed below:

Component	PCR reaction volume	
	10 µL	20 µL
EpiMelt Master Mix 2×	5 µL	10 µL
Primer 1**	0.1 - 1 µM*	0.1 - 1 µM*
Primer 2**	0.1 - 1 µM*	0.1 - 1 µM*
DNA template	3 ng - 1 µg	3 ng - 1 µg
Sterile Water	Up to 10 µL	Up to 20 µL

Table 3. Components of the PCR mix.
**: Recommended for standard real-time PCR.*
***: Final concentration in the reaction mixture.*

- Gently vortex the samples and briefly centrifuge to ensure that the content is collected at the bottom of the tube.
- Place the tubes in the thermocycler and start the PCR program.

Example of an amplification profile:

Step	Temperature	Time
Initial denaturation	95°C	10 min
35-50 cycles	95°C 50-68°C 72°C	15-30 s 30-60 s 15-60 s*

Table 4. Example of an amplification profile.
**: Depending on the length of the PCR product, for products >500 bp 1 min PCR product melting analysis is recommended.*

Recommended ROX mixture

HiROX (0.6-1 µL per 50 µL of total reaction volume): Applied Biosystems: 7000, 7300, 7700, 7900HT Fast, StepOne, StepOnePlus.

LoROX (0.6-1 µL per 50 µL of total reaction volume): Applied Biosystems: 7500, Stratagene: Mx3000P, Mx3005P, Mx4000P.

Ordering information

MethylDetect offers a number of EpiMelt Methylation Detection Assays targeting specific genomic regions. For a complete overview of the products and ordering information, please visit www.MethylDetect.com

Supplementary information

License disclaimer

For patent license limitations for individual products, please refer to www.MethylDetect.com

Regulatory disclaimer

For Life Science research only. Not for use in diagnostic procedures.
EvaGreen® is a registered trademark of Biotium Inc.

Safety data sheet

Please follow the instructions in the safety data sheet (SDS) at www.MethylDetect.com

Contact and support

Please refer to www.MethylDetect.com