

EZ qPCR/HRM PreMix

Cat. Nos. E2005 (100 Reactions)

E2006 (400 Reactions)

Storage: -20 °C



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The Beauty of Science is to Make Things Simple

Product Information

Features:

- Site-specific DNA methylation quantification using real-time PCR and HRM analysis
- Ideal for the robust amplification of *bisulfite-treated* DNA for methylation detection
- Strong fluorescent signal for real-time and quantitative PCR assays
- Applicable for other HRM analyses such as SNP detection and genotyping

Description:

The EZ qPCR/HRM PreMix product contains all the reagents needed to perform quantitative PCR and high-resolution melt (HRM) analysis of DNA methylation. It features a hot-start DNA polymerase and buffer system optimized for the amplification of *bisulfite-treated* DNA. It also includes an intense dsDNA-specific fluorescent dye for sensitive DNA quantification. The EZ qPCR/HRM PreMix allows measurement of DNA methylation status, SNP analysis, and genotyping. The product can also be used for conventional quantitative PCR assays.

Product Contents:

	E2005 (100 Rxns.)	E2006 (400 Rxns.)	Storage Temp.
EZ qPCR/HRM PreMix (2X)	1 ml	4 x 1 ml	-20 °C
DNase/RNase-Free H ₂ O	2 x 1 ml	10 ml	Room Temp.
DNA standard & primer set	✓	✓	-20 °C

Storage:

Store at -20 °C for up to 12 months. Minimize exposure to light. Avoid repeated freeze/thawing of reagents.

Suggested Reaction Setup (20 µl):

Reagent	Volume	Final conc.
EZ qPCR/HRM PreMix (2X)	10 µl	1 X
Forward Primer (10 µM)	Variable	0.3 to 1 µM
Reverse Primer (10 µM)	Variable	0.3 to 1 µM
Template DNA	Variable	< 100 ng/20 µl
ddH ₂ O	to 20 µl	–
Total volume	20 µl	

Note: The final concentration of MgCl₂ in the reaction (above) is 1.75 mM. If required, scale reaction reagent volumes accordingly to optimize the MgCl₂, primer, and/or template concentrations.

Primer Design Recommendations:

Primers should be between 25 and 35 bases long and amplify sequences of 200-400 bp. Primer sequences should include as many guanines (Gs) as possible while avoiding CG dinucleotides. Predicted primer *T_m*s should be above 50°C (ideally 54-59°C).

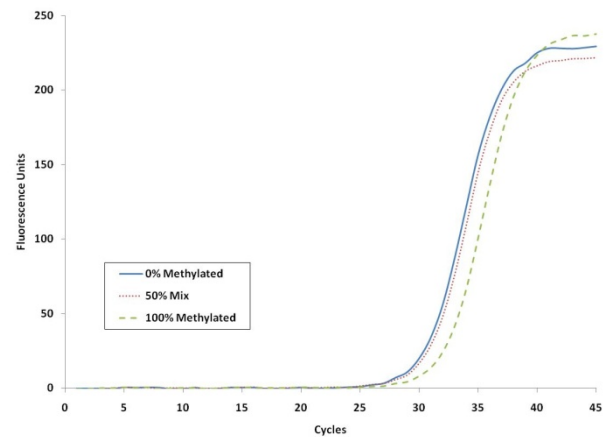
Instrumentation:

The product has been optimized for LightCycler® 480 (Roche) & CFX96™ (Bio-Rad), but real-time PCR and HRM can be performed on any instrument that has HRM software and optical scanners that don't require passive reference dye.

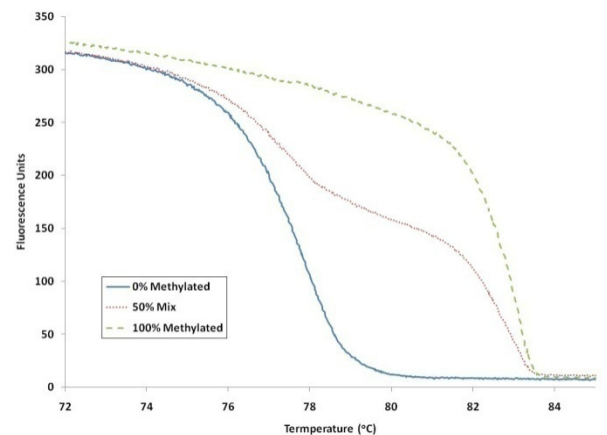
Suggested Conditions For PCR:

Initial denaturation	95 °C	10 min.
Denaturation	94 to 96 °C	30 sec.
Annealing	Variable	60 sec.
Extension	72 °C	60 sec. for ≤ 1kb*
	40-45 Cycles	
Final extension	72 °C	2 min.
Hold	4 °C	> 4 min.

***Note:** Add 15 to 30 seconds to the extension time for each additional kb over 1 kb. Make adjustments to the temperature if necessary.



Real-Time Amplification Plot. This figure shows robust real-time amplification of 10 ng of bisulfite-converted human DNA using the EZ qPCR/HRM PreMix and primers designed to amplify a region of the *MGMT* promoter.



High Resolution Melt Plot. This figure shows raw data from the high resolution melt that immediately followed the amplification seen above. It illustrates the difference in melt profiles associated with different methylation states.

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Related Products:

Product Name	Size	Catalog number
EZ DNA Methylation-Direct™ qPCR/HRM Kit Contains: EZ qPCR/HRM PreMix (100 Rxns.) EZ DNA Methylation-Direct™ Kit (50 Preps) Human Methylated & Non-Methylated DNA Set (1) Control primer set (1)	100 Rxns.	D5300
ZymoTaq™ DNA Polymerase	50 Rxns.	E2001
	200 Rxns.	E2002
ZymoTaq™ PreMix	50 Rxns.	E2003
	200 Rxns.	E2004

Also Available:

Product Name	Size	Catalog number
EZ DNA Methylation™ Kit	50	D5001
	200	D5002
	2 x 96	D5003
	2 x 96	D5004
EZ DNA Methylation-Gold™ Kit	50	D5005
	200	D5006
	2 x 96	D5007
	2 x 96	D5008
EZ DNA Methylation-Direct™ Kit	50	D5020
	200	D5021
	2 x 96	D5022
	2 x 96	D5023
EZ DNA Methylation-Startup™ Kit	1 Kit	D5024
EZ Bisulfite DNA Clean-up Kit™	50	D5025
	200	D5026
	2 x 96	D5027
	2 x 96	D5028
Universal Methylated DNA Standard	1 set	D5010
Universal Methylated Human DNA Standard	1 set	D5011
Universal Methylated Mouse DNA Standard	1 set	D5012
Human HCT116 DKO Methylation Standards	1 set	D5014
Human HCT116 DKO Non-methylated DNA Standard	5 µg	D5014-1
Human HCT116 DKO Methylated DNA Standard	5 µg	D5014-2
Bisulfite Converted Universal Methylated Human DNA Standard	1 set	D5015
<i>E. coli</i> Non-methylated Genomic DNA	5 µg	D5016
Methylated-DNA IP Kit	10	D5101
ChIP DNA Clean & Concentrator™	50	D5201
	50	D5205
Anti-5-Methylcytosine Monoclonal Antibody (clone 10G4)	50 µg 200 µg	A3001-50 A3001-200
CpG Methylase (M.SssI)	200 units	E2010
	400 units	E2011

Trademarks and Disclaimers:

Trademarks: ZymoTaq™ (Zymo Research Corp.); LightCycler® (Roche Group); CFX96™ (Bio-Rad Laboratories, Inc.)

This product is for research use only and should only be used by trained professionals. Wear protective gloves and eye protection. Follow the safety guidelines and rules enacted by your research institution or facility.

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