

# QuestTaq™ qPCR PreMix

Cat. Nos. E2052 (50 Rxns.)

E2053 (200 Rxns.)

Storage: -20°C



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The Beauty of Science is to Make Things Simple

## Product Information

### Features:

- One-tube premixed reagents for real-time PCR analysis
- Ideal for robust, non-biased amplification of 5mC, 5hmC, and g5hmC modified DNA
- Includes a strong fluorescent dye (SYTO® 9) for real-time and quantitative PCR applications
- Compatible with HRM-based procedures

### Description:

QuestTaq™ qPCR PreMix contains all reagents necessary to perform *unbiased* quantitative PCR with DNA having 5-methylcytosine (5mC), 5-hydroxymethylcytosine (5hmC), and glucosyl-5-hydroxymethylcytosine (g5hmC) composition. It features a unique DNA polymerase and optimized buffer system for amplification of DNA having modified/unmodified cytosines. It also includes an intense dsDNA-specific fluorescent dye (SYTO® 9) for sensitive/precise DNA detection. QuestTaq™ qPCR PreMix is ideal for locus-specific 5-hydroxymethylcytosine detection when used in conjunction with the Quest 5-hmC Detection Kit™ (Cat. Nos. D5410 & D5411 from Zymo Research) and the Quest 5-hmC Detection Kit™-Lite (D5415 & D5416).

### Product Contents:

	E2052 (50 Rxns.)	E2053 (200 Rxns.)	Conc.	Storage Temp.
QuestTaq™ qPCR PreMix*	500 µl	4 x 500 µl	2X	-20°C
DNase/RNase- Free H <sub>2</sub> O	1 ml	2 x 1 ml	-	Room Temp.

\*2X QuestTaq™ qPCR PreMix contains QuestTaq™ DNA polymerase, dNTPs, MgCl<sub>2</sub>, SYTO® 9 dye, and reaction buffer. SYTO® 9 dye has excitation/emission maxima ~480 nm/520 nm, respectively.

### Storage:

QuestTaq™ qPCR PreMix should be stored in the dark between -20 °C to -80 °C for maximum performance. Performance is guaranteed for 1 year from the time of receipt.

### Enzyme Concentration:

Reaction conditions at 1X (20 µl total volume) will contain 2 units of QuestTaq™ DNA polymerase.

### Unit Definition:

One unit (U) enzyme of QuestTaq™ DNA polymerase is defined as the amount of enzyme required for the incorporation of 10 nmoles dNTPs into an acid-insoluble form in 30 minutes at 72 °C.

### Enzyme Property:

QuestTaq™ DNA polymerase has 5'→3' exonuclease activity but does not have 3'→5' exonuclease activity. It leaves A-overhangs and is suitable for TA cloning.

### Instrumentation:

Compatible with real-time PCR instruments that don't require a passive reference dye (e.g., LightCycler® 480 (Roche), CFX96™ (Bio-Rad), etc.)

### Suggested Reaction Setup:

Reagent	Volume	Final concentration
QuestTaq™ qPCR PreMix	10 µl	1X
Primers (forward/reverse)	Variable	0.3 to 1 µM each
Template	Variable	< 20 ng/20 µl
ddH <sub>2</sub> O	to 20 µl	-
Total volume	20 µl	

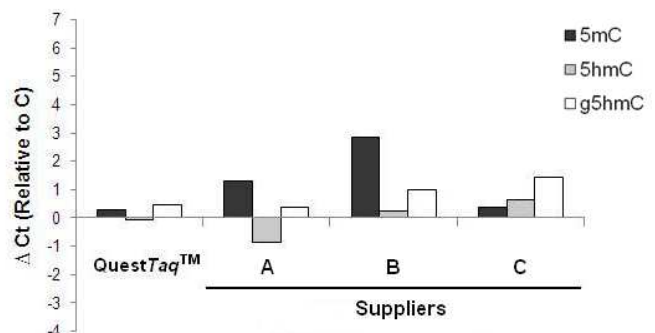
**Note:** If required, scale reaction reagent volumes accordingly to accommodate desired primer and/or template concentrations. It is recommended to setup reactions on ice for consistent results between multiple sample replicates.

### Suggested Conditions for qPCR:

Set the real-time PCR instrument to excitation and emission wavelengths of ~480 nm/520 nm, respectively.

<b>Initial Denaturation</b>	95 °C	1 min.
<b>Denaturation</b>	94 to 96 °C	30 sec.
<b>Annealing</b>	Variable	30-40 sec.
<b>Extension</b>	72 °C	30-60 sec. for ≤ 1kb*
<b>30-40 Cycles</b>		
<b>Final Extension</b>	72 °C	7 min.
<b>Hold</b>	4 °C	> 4 min.

\*Note: Add 15 to 30 seconds to the extension time for each additional kb >1 kb. Make adjustments to the annealing temperature as necessary.



### QuestTaq™ qPCR PreMix Analysis:

QuestTaq™ Polymerase yields the least cycle threshold variation ( $\Delta C_t$ ) versus the polymerases from Suppliers A, B, and C when amplifying DNA with modified cytosines. The figure shows  $\Delta C_t$  values from real-time PCR data generated using a LightCycler® 480 instrument. Equivalent amounts of template DNA having modified cytosines (5mC, 5hmC, and g5hmC) were compared to unmodified template (*post*-amplification) using QuestTaq™ qPCR PreMix or the polymerases from Suppliers A, B, and C.

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**Related Products:**

Product Name	Size	Catalog No.
<b>Quest 5-hmC Detection Kit™</b>	25 Preps.	D5410
	50 Preps.	D5411
<b>Quest 5-hmC Detection Kit™-Lite</b>	25 Preps.	D5415
	50 Preps.	D5416
<b>Quest Taq™ PreMix</b>	50 Rxns.	E2050
	200 Rxns.	E2051
<b>Human Matched DNA Set</b>	2 x 5 µg	D5018
<b>Mouse <sup>5</sup>hmC &amp; <sup>5</sup>mC DNA Set</b>	4 x 5 µg	D5019
<b>5-Methylcytosine &amp; 5-Hydroxymethylcytosine DNA Standard Set</b>	3 x 2 µg	D5405
<b>DNA Degradase™</b>	500 units	E2016
	2,000 units	E2017
<b>DNA Degradase Plus™</b>	250 units	E2020
	1,000 units	E2021
<b>5-hmC Glucosyltransferase</b>	100 units	E2026
	200 units	E2027
<b>5-Hydroxymethyl dCTP [100 mM]</b>	10 µmol	D1045
<b>5-Hydroxymethylcytosine dNTP Mix [10 mM]</b>	2.5 µmol	D1040
<b>5-Methyl dCTP [10 mM]</b>	1 µmol	D1035
<b>5-Methylcytosine dNTP Mix [10 mM]</b>	2.5 µmol	D1030

**Also Available:**

Product Name	Size	Catalog No.
<b>OneStep qMethyl™ Kit</b>	1 x 96	D5310
<b>OneStep qMethyl™-Lite</b>	1 x 96	D5311
<b>Zymo Taq™ DNA Polymerase</b>	50	E2001
	200	E2002
<b>Zymo Taq™ PreMix</b>	50	E2003
	200	E2004
<b>EZ DNA Methylation™ Kit</b>	50	D5001
	200	D5002
	2 x 96	D5003
	2 x 96	D5004
<b>EZ DNA Methylation-Gold™ Kit</b>	50	D5005
	200	D5006
	2 x 96	D5007
	2 x 96	D5008
<b>EZ DNA Methylation-Direct™ Kit</b>	50	D5020
	200	D5021
	2 x 96	D5022
	2 x 96	D5023
<b>EZ DNA Methylation-Startup™ Kit</b>	1 Kit	D5024
<b>EZ Bisulfite DNA Clean-up Kit™</b>	50	D5025
	200	D5026
	2 x 96	D5027
	2 x 96	D5028
<b>Universal Methylated DNA Standard</b>	1 set	D5010
<b>Universal Methylated Human DNA Standard</b>	1 set	D5011
<b>Universal Methylated Mouse DNA Standard</b>	1 set	D5012
<b>Human HCT116 DKO Methylation Standards</b>	1 set	D5014
<b>Human HCT116 DKO Non-methylated DNA Standard</b>	5 µg	D5014-1
<b>Human HCT116 DKO Methylated DNA Standard</b>	5 µg	D5014-2
<b>Bisulfite Converted Universal Methylated Human DNA Standard</b>	1 set	D5015
<b>E. coli Non-methylated Genomic DNA</b>	5 µg	D5016
<b>Methylated-DNA IP Kit</b>	10	D5101
<b>ChIP DNA Clean &amp; Concentrator™</b>	50	D5201
	50	D5205
<b>Anti-5-Methylcytosine Monoclonal Antibody (clone 10G4)</b>	50 µg 200 µg	A3001-50 A3001-200
<b>CpG Methylase (M.SssI)</b>	200 units	E2010
	400 units	E2011

**Trademarks and Disclaimers:**

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The Polymerase Chain Reaction (PCR) process is covered by U.S. Pat. Nos. 4,683,195 and 4,683,202 assigned to Hoffmann-La Roche. Patents pending in other countries. No license under these patents to use the PCR process is conveyed expressly or by implication to the purchaser by the purchase of Zymo Research's OneStep qMethyl™ Kit. Further information on purchasing licenses to practice the PCR process can be obtained from the director of Licensing at Applied Biosystems, 850 Lincoln Centre Drive, Foster City, California 94404 or at Roche Molecular Systems, Inc., 1145 Atlantic Avenue, Alameda, California 94501.

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