

QuestTaq™ PreMix

Cat. Nos. **E2050** (50 Rxns.)
E2051 (200 Rxns.)



Storage: -20°C

Product Information

Features:

- Premixed reagents for one-tube PCR
- Ideal for robust, non-biased amplification of 5mC, 5hmC, and g5hmC modified DNA
- Ideal for real-time, quantitative, and end-point analyses
- Compatible with a range of fluorescent dyes for use in real-time PCR

Description:

QuestTaq™ PreMix is supplied as a convenient 2X concentrated “master mix” containing all the reagents (i.e., dNTPs, MgCl₂, and enhancers) necessary for robust PCR with little or no by-product formation. The QuestTaq™ PreMix has been optimized for the non-biased amplification of cytosine, 5-methylcytosine (5mC), 5-hydroxymethylcytosine (5hmC), and glucosyl-5-hydroxymethylcytosine (g5hmC) containing DNA, ensuring high yield amplification across a wide range of templates. The QuestTaq™ PreMix differs from QuestTaq™ qPCR PreMix (Cat. Nos. E2052 & E2053 from Zymo Research) in that it excludes SYTO® 9 dye from the PreMix solution, making it compatible with real-time and quantitative PCR with fluorescent dyes of the researcher’s choosing.

Product Contents:

| | E2050 (50 Rxns.) | E2051 (200 Rxns.) | Conc. | Storage Temp. |
|--|----------------------------|-----------------------------|--------------|----------------------|
| QuestTaq™ PreMix* | 500 µl | 4 x 500 µl | 2X | -20°C |
| DNase/RNase-Free H₂O | 1 ml | 2 x 1 ml | - | Room Temp. |

*2X QuestTaq™ Premix contains QuestTaq™ DNA polymerase, dNTPs, MgCl₂, and reaction buffer.

Storage:

QuestTaq™ PreMix should be stored 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 nmol 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.

Proposed Reaction Setup:

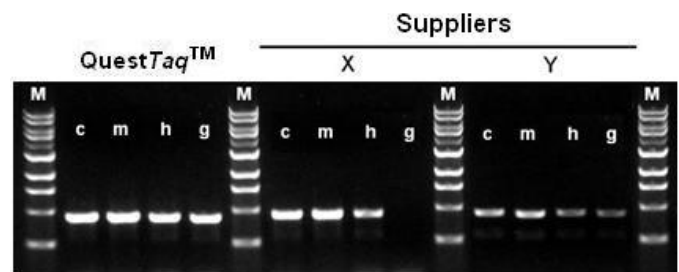
| Reagent | Volume | Final concentration |
|---------------------------|----------|---------------------|
| QuestTaq™ PreMix | 10 µl | 1X |
| Primers (forward/reverse) | Variable | 0.3 to 1 µM each |
| Template | Variable | < 20 ng/20 µl |
| ddH ₂ O | to 20 µl | - |
| Total volume | 20 µl | |

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 PCR:

| | | |
|-----------------------------|-----------|------------------------------|
| Initial denaturation | 95°C | 1 min. |
| Denaturation | 94 – 96°C | 30 sec. |
| Annealing | Variable | 30 - 40 sec. |
| Extension | 72°C | 30 sec. - 1 min. for ≤ 1 kb* |
| 25-30 Cycles | | |
| Final extension | 72°C | 7 min. |
| Hold | 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 and number of cycles as necessary.



QuestTaq™ polymerase consistently yields robust amplicons from DNA templates having modified/unmodified cytosines. The figure shows the level (intensity) of an ~900 bp product generated from DNA templates using QuestTaq™ PreMix or the polymerases from Suppliers X and Y. In each case, PCR products were separated in a 0.8% (w/v) agarose/TAE/EtBr gel. Lanes correspond to amplicons from template DNA containing: unmodified cytosine (c), 5-methylcytosine (m), 5-hydroxymethylcytosine (h), or glucosyl-5-hydroxymethylcytosine (g). (M) is a 1 kb DNA Marker (Zymo Research, Cat. No. M5006-50).

ZYMO RESEARCH CORP.

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Related Products:

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

Also Available:

| Product Name | Size | Catalog No. |
|---|-------------------------------|----------------------------------|
| <i>OneStep</i> qMethyl™ Kit | 1 x 96 | D5310 |
| <i>OneStep</i> qMethyl™-Lite | 1 x 96 | D5311 |
| Zymo Taq™ DNA Polymerase | 50 200 | E2001 E2002 |
| Zymo Taq™ PreMix | 50 200 | E2003 E2004 |
| EZ DNA Methylation™ Kit | 50 200 2 x 96 2 x 96 | D5001 D5002 D5003 D5004 |
| EZ DNA Methylation-Gold™ Kit | 50 200 2 x 96 2 x 96 | D5005 D5006 D5007 D5008 |
| EZ DNA Methylation-Direct™ Kit | 50 200 2 x 96 2 x 96 | D5020 D5021 D5022 D5023 |
| EZ DNA Methylation-Startup™ Kit | 1 Kit | D5024 |
| EZ Bisulfite DNA Clean-up Kit™ | 50 200 2 x 96 2 x 96 | D5025 D5026 D5027 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 50 | D5201 D5205 |
| Anti-5-Methylcytosine Monoclonal Antibody (clone 10G4) | 50 µg 200 µg | A3001-50 A3001-200 |
| CpG Methylase (M.Sssl) | 200 units 400 units | E2010 E2011 |

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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.

Version 1.0.1

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