PerfeCTa® qPCR FastMix®, UNG, Low ROX™

Cat No. 95078-012 Size: 1250 x 20-µL reactions (10 x 1.25 mL)
95078-05K 5000 x 20-µL reactions (1 x 50 mL)

Store at -25°C to -15ºC protected from light

Description

PerfeCTa qPCR FastMix, UNG, Low ROX is a 2X concentrated, ready-to-use reaction cocktail that contains all components, except primers, probe(s), and template for real-time quantitative PCR on Applied Biosystems 7500, 7500 Fast, ViiA™ 7 or Stratagene MX series of real-time PCR systems. The proprietary buffer and stabilizers have been specifically optimized to deliver maximum PCR efficiency, sensitivity, and robust fluorescent signal with TaqMan® or TaqMan MGB probe chemistry when using rapid PCR cycle times and reduced reaction volumes. This affords greater reagent economy and laboratory throughput on conventional or rapid ramp rate qPCR systems. The enhanced specificity of this FastMix suppresses cross-reactivity between homologous sequences, improving detection and discrimination in SNP applications. A key component of this FastMix is AccuFast™ Taq DNA polymerase. This hot-start Taq contains a proprietary mixture of monoclonal antibodies that bind to the polymerase and keep it inactive prior to the initial PCR denaturation step (> 48 hours at room temperature). Similar to our AccuStart™ Taq DNA polymerase, these antibodies are irreversibly inactivated during the initial PCR denaturation step. However, unlike other antibody hot-start polymerases, activation of AccuFast Taq is instantaneous at 95ºC. Rapid recovery of fully active, unmodified Taq DNA polymerase is critical for efficient extension kinetics. Replication of fragments up to 200 bp is complete in less than 20s at 60ºC. Additionally, the dNTP mix in this FastMix contains dUTP in place of dTTP. Inclusion of uracil-N-glycosylase (UNG) prevents amplification of carry-over contamination from previous dU-containing PCRs.

Instrument Compatibility

Different real-time PCR systems employ different strategies for the normalization of fluorescent signals and correction of well-to-well optical variations. It is critical to match the appropriate qPCR reagent to your specific instrument. PerfeCTa qPCR FastMix, UNG, Low ROX provides seamless integration on the Applied Biosystems 7500, 7500 Fast, ViiA 7, or Stratagene MX series of real-time PCR systems. Please consult the following table, or visit our web site at www.quantabio.com to find the optimal kit for your instrument platform.

Reagent	Cat Nos	Compatible Real-Time PCR Systems
PerfeCTa qPCR FastMix, UNG, ROX	95077-012, 95077-05K	Applied Biosystems 7000, 7300, 7700, 7900, 7900HT, 7900HT Fast, StepOne™, StepOnePlus™
PerfeCTa qPCR FastMix, UNG, Low ROX	95078-012, 95078-05K	Applied Biosystems 7500, 7500 Fast, ViiA™ 7 Stratagene MX4000™, MX3005P™, MX3000P™
PerfeCTa qPCR FastMix, UNG	95076-012, 95076-05K	Bio-Rad CFX96™, CFX384™,iCycler iQ®, iQ™5, MyiQ™ Opticon™, MiniOpticon™, Chromo4™ Cepheid Smart Cycler®; Qiagen/Corbett Rotor-Gene® Eppendorf Mastercycler® ep realplex Roche Applied Science LightCycler® 480

Components

PerfeCTa qPCR FastMix, UNG, Low ROX (2X): 2X reaction buffer containing optimized concentrations of $MgCl_2$, dNTPs (dATP, dCTP, dGTP, dUTP), AccuFast Taq DNA Polymerase, UNG, ROX Reference Dye and stabilizers.

Storage and Stability

Store components in a constant temperature freezer at -25°C to -15°C protected from light upon receipt.
For lot specific expiry date, refer to package label, Certificate of Analysis or Product Specification Form.

Guidelines for qPCR:

- The design of highly specific primers and probes is a critical parameter for successful real-time PCR. The use of computer aided primer design programs is encouraged in order to minimize the potential for internal secondary structure and complementation at 3'-ends within each primer, the primer pair, and primer/probe combinations. For best results, amplicon size should be limited to 65 - 150 bp. Optimal results may require titration of primer concentration between 100 and 900 nM. A final concentration of 300-400 nM each primer and 100-150 nM probe is effective for most applications. However, increasing the concentration of the primer that initiates synthesis of the target strand that is complementary to the probe can improve fluorescent signal for some primer/probe systems. Validate the efficacy and efficiency of any primer/probe set under fast cycling and/or rapid ramp rate protocols before use in qPCR studies.
- Preparation of a reaction cocktail is recommended to reduce pipetting errors and maximize assay precision. Assemble the reaction cocktail with all required components except sample template (genomic DNA or cDNA) and dispense equal aliquots into each reaction tube. Add the DNA template to each reaction as the final step. Addition of samples as 5-µL volumes will improve assay precision.
- Suggested input quantities of template are: cDNA corresponding to 1 pg to 1 µg of total RNA; 100 pg to 1 µg genomic DNA
- After sealing each reaction, vortex gently to mix contents. Centrifuge briefly to collect components at the bottom of the reaction tube.

Reaction Assembly

Component	Volume for 20-µL rxn.	Final Concentration
PerfeCTa qPCR FastMix, UNG, Low ROX (2X)	10 µL	1x
Forward primer	variable	300 – 400 nM
Reverse primer	variable	300 – 400 nM
Probe	variable	100 – 150 nM
Nuclease-free water	variable	
Template	5 µL	variable
Final Volume (µL)	20 µL	

Reaction Protocol

Incubate complete reaction mix in a real-time thermal detection system as follows:

UNG incubation	45ºC, 2 min (optional)
Initial denaturation:	95ºC, 30s (see notes below)
PCR cycling (30-45 cycles:)	95ºC, 1 to 3s
	55 – 65ºC, 15 to 30 s (collect and analyze data)

Optimal extension time may be amplicon specific. Use 20s at 60ºC as a general starting point. The minimum required extension time will vary for different instrument platforms. Consult your instrument instructions.

Full activation of AccuFast Taq DNA polymerase is instantaneous at 95ºC; however, optimal initial denaturation time is *template dependent* and will affect qPCR efficiency and sensitivity. Amplification of genomic DNA or supercoiled plasmid DNA targets may require 5 to 10 min at 95ºC to fully denature and fragment the template. This minimizes the potential for renaturation of long fragments and/or repetitive sequence regions that can impair PCR. Short double-stranded DNA template (PCR product) or single-stranded DNA template, such as cDNA template generated using the qScript™ cDNA Synthesis Kit or qScript cDNA SuperMix, require as little as 1s at 95ºC. Oligo-dT primed cDNA using an RNase H deficient reverse transcriptase may require 2-3 minutes. Use 30s at 95ºC as a general starting point.

Quality Control

Kit components are free of contaminating DNase and RNase. PerfeCTa qPCR FastMix, UNG, Low ROX is functionally tested in qPCR. Kinetic analysis must demonstrate linear resolution over six orders of dynamic range ($r^2 > 0.995$) and a PCR efficiency > 90%.

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