PerfeCTa® qPCR SuperMix, UNG, Low ROX™

Cat. No.	95066-100	Size:	100 x 50-µL reactions (2 x 1.25 mL)	**Store at -20ºC**
	95066-500		500 x 50-µL reactions (10 x 1.25 mL)	
	95066-02K		2000 x 50-µL reactions (1 x 50 mL)	

Description

PerfeCTa qPCR SuperMix, 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. The enhanced specificity of this supermix suppresses cross-reactivity between homologous sequences, improving detection and discrimination in SNP applications. A key component of this supermix is AccuStart™ Taq DNA polymerase, which contains monoclonal antibodies that bind to the polymerase and keep it inactive prior to the initial PCR denaturation step. Upon heat activation (2 minutes at 95ºC), the antibodies denature irreversibly, releasing fully active, unmodified Taq DNA polymerase. This enables specific and efficient primer extension with the convenience of room temperature reaction assembly. Additionally, the dNTP mix in this SuperMix 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 SuperMix, 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. A full line of qPCR SuperMixes without dUTP and UNG are also available.

Reagent	Cat Nos	Compatible Real-Time PCR Systems
PerfeCTa qPCR SuperMix, UNG, ROX	95065-100, 95065-500, 95065-02K	Applied Biosystems 7000, 7300, 7700, 7900, 7900HT, 7900HT Fast, StepOne™, StepOnePlus™
PerfeCTa qPCR SuperMix, UNG, Low ROX	95066-100, 95066-500, 95066-02K	Applied Biosystems 7500, 7500 Fast, ViiA™ 7 Stratagene MX4000™, MX3005P™, MX3000P™
PerfeCTa qPCR SuperMix, UNG	95064-100, 95064-500, 95064-02K	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 SuperMix, UNG, Low ROX (2X): 2X reaction buffer containing optimized concentrations of $MgCl_2$, dNTPs (dATP, dCTP, dGTP, dUTP), AccuStart Taq DNA Polymerase, UNG, ROX Reference Dye (for 580-585 nm excitation), and stabilizers.

Storage and Stability

PerfeCTa qPCR SuperMix, UNG, Low ROX is stable for 1 year when stored in a constant temperature freezer at -20ºC. For convenience, it may be stored unfrozen at +2 to +8ºC for up to 6 months.

Repeated freezing and thawing of the supermix is not recommended. However, the product demonstrated no loss of performance after 20 freeze-thaw cycles or 2 months at +20ºC.

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. PerfeCTa qPCR SuperMix, UNG, Low ROX can readily amplify fragments between 400 and 500 bp; however, for best results, amplicon size should be limited to 65 - 200 bp. Optimal results may require titration of primer concentration between 100 and 900 nM. A final concentration of 300 nM each primer and 100 to 250 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.

Guidelines for qPCR continued:

- 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 to 10-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 50-µL rxn.	Final Concentration
PerfeCTa qPCR SuperMix, UNG, Low ROX (2X)	25 µL	1x
Forward primer	variable	100 – 900 nM
Reverse primer	variable	100 – 900 nM
Probe	variable	100 – 250 nM
Nuclease-free water	variable	
Template	5 – 10 µL	variable
Final Volume (µL)	50 µL	

Note: For smaller reaction volumes (i.e. 25-µL reactions), scale all components proportionally.

Reaction Protocol

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

UNG incubation	45ºC, 5 min (optional)
Initial denaturation:	95ºC, 2 to 3 min
PCR cycling (30-45 cycles:)	95ºC, 10 to 15 s
	55 – 65ºC, 30 to 45 s (collect and analyze data)

Full activation of AccuStart Taq DNA polymerase occurs within 30 seconds at 95ºC. Initial denaturation times greater than 3 minutes are not required. However, amplification of genomic DNA or supercoiled plasmid DNA targets may benefit from a prolonged initial denaturation step (5-10 min) to fully denature and fragment the template. This minimizes the potential for renaturation of long fragments and/or repetitive sequence regions that can impair replication of the target sequence by the PCR process.

Some primer sets may require a 3-step cycling protocol for optimal performance. Optimal annealing temperature and time may need to be empirically determined for any given primer set. A 68ºC extension step of 30 seconds is suitable for most applications. However, amplicons greater than 200 bp may require longer extension times.

Quality Control

Kit components are free of contaminating DNase and RNase. PerfeCTa qPCR SuperMix, 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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