PerfeCTa™ SYBR® Green SuperMix, UNG, ROX

Cat No.	Size:		Store at -20°C protected from light
95069-100	100 x 50 µL reactions (2 x 1.25 mL)		
95069-500	500 x 50 µL reactions (10 x 1.25 mL)		
95069-02K	2000 x 50 µL reactions (1 x 50 mL)		

Description

PerfeCTa SYBR Green SuperMix, UNG, ROX is a 2X concentrated, ready-to-use reaction cocktail that contains all components, except primers and template for real-time quantitative PCR on Applied Biosystems 7000, 7300, 7700, or 7900 instruments. The proprietary buffer and stabilizers have been optimized exclusively for SYBR Green I qPCR to deliver maximum PCR efficiency, sensitivity, and robust fluorescent signal. Highly specific amplification is crucial to successful qPCR with SYBR Green I technology because this dye binds to and detects any dsDNA generated during amplification. 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 95°C), 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 SYBR Green SuperMix, UNG, ROX provides seamless integration on the Applied Biosystems 7000, 7300, 7700, 7900, or 7900HT. Please consult the following table, or visit our web site at www.quantabio.com, to find the optimal kit for your instrument platform. SYBR Green SuperMixes without dUTP and UNG are also available.

Reagent	Cat Nos	Compatible Real-Time PCR Systems
PerfeCTa SYBR Green SuperMix, UNG, ROX	95069-100, 95069-500, 95069-02K	Applied Biosystems 7000, 7300, 7700, 7900, 7900HT
PerfeCTa SYBR Green SuperMix, UNG, Low ROX	95070-100, 95070-500, 95070-02K	Applied BioSystems 7500 Stratagene MX4000™, MX3005P™, MX3000P™
PerfeCTa SYBR Green SuperMix, UNG, for iQ	95067-100, 95067-500, 95067-02K	Bio-Rad iCycler iQ®, iQ™5, MyiQ™
PerfeCTa SYBR Green SuperMix, UNG	95068-100, 95068-500, 95068-02K	Bio-Rad / MJ Opticon™, MiniOpticon™, Chromo4™ Cepheid Smart Cycler®; Corbett Rotor-Gene™ Eppendorf Mastercycler® ep realplex Roche Applied Science LightCycler® 480

Components

PerfeCTa SYBR Green SuperMix, UNG, ROX (2X): 2X reaction buffer containing optimized concentrations of $MgCl_2$, dNTPs (dATP, dCTP, dGTP, dUTP), AccuStart Taq DNA Polymerase, UNG, SYBR Green I dye, ROX Reference Dye and stabilizers.

Storage and Stability

PerfeCTa SYBR Green SuperMix, UNG, ROX is stable for 1 year when stored in a constant temperature freezer at -20°C, protected from light. For convenience, it may be stored unfrozen at +2 to +8°C 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 +20°C.

Guidelines for SYBR Green qPCR:

- The design of highly specific primers is the single most important parameter for successful real-time PCR with SYBR Green I dye. 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 and the primer pair. PerfeCTa SYBR Green SuperMix, UNG, ROX can readily amplify fragments between 400 and 500 bp; however, for best results, amplicon size should be limited to 80 - 200 bp. Optimal results may require titration of primer concentration between 100 and 500 nM. A final concentration of 300 nM for each primer is effective for most reactions.
- 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 100 ng of total RNA; 100 pg to 100 ng 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 SYBR Green SuperMix, UNG, ROX (2X)	25 µL	1x
Forward primer	variable	100 – 500 nM
Reverse primer	variable	100 – 500 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
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)
Melt Curve (dissociation stage)	Refer to instrument instructions (optional)

Full activation of AccuStart Taq DNA polymerase occurs within 30 seconds at 95ºC. Initial denaturation times greater than 3 minutes are not recommended. 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 68 to 72ºC extension step of 30 seconds is suitable for most applications. However, amplicons greater than 200 bp may require longer extension times. The use of an elevated temperature (80ºC) for data collection is not recommended. While this technique can be used to mask the detection of primer-dimer and/or other non-specific products, it does little to improve assay specificity or sensitivity and is not a substitute for effective primer design.

Quality Control

Kit components are free of contaminating DNase and RNase. PerfeCTa SYBR Green SuperMix, UNG, 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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