Quanta BIOSCIENCES™

PerfeCTa™ SYBR® Green FastMix™

Cat No.		Size:		
Cat No.	95072-250	Size:	250 x 20 µL reactions (2 x 1.25 mL)	**Store at -20°C protected from light**
	95072-012		1250 x 20 µL reactions (10 x 1.25 mL)	
	95072-05K		5000 x 20 µL reactions (1 x 50 mL)	

Description

PerfeCTa SYBR Green FastMix is a 2X concentrated, ready-to-use reaction cocktail that contains all components, except primers and template for real-time quantitative PCR systems that do not require an internal reference dye. This unique combination of proprietary buffer, stabilizers, and AccuFast™ Taq DNA polymerase deliver maximum PCR efficiency, sensitivity, specificity and robust fluorescent signal using fast, or conventional, cycling protocols with SYBR Green qPCR.

Highly specific amplification is crucial to successful qPCR with SYBR Green I dye technology because this dye binds to and detects any dsDNA generated during amplification. AccuFast Taq DNA polymerase is a key component of this reaction mix. 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 95°C. 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 60°C.

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 FastMix does not contain an internal reference dye. 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 SYBR Green FastMix, ROX	95073-250, 95073-012, 95073-05K	Applied Biosystems 7000, 7300, 7700, 7900, 7900HT, StepOne™, StepOnePlus™
PerfeCTa SYBR Green FastMix, Low ROX	95074-250, 95074-012, 95074-05K	Applied Biosystems 7500 Stratagene MX4000™, MX3005P™, MX3000P™
PerfeCTa SYBR Green FastMix for iQ	95071-250, 95071-012, 95071-05K	Bio-Rad iCycler iQ®, iQ™5, MyiQ™
PerfeCTa SYBR Green FastMix	95072-250, 95072-012, 95072-05K	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 FastMix (2X): 2X reaction buffer containing optimized concentrations of $MgCl_2$, dNTPs (dATP, dCTP, dGTP, dTTP), AccuFast Taq DNA Polymerase, SYBR Green I dye, and stabilizers.

Storage and Stability

PerfeCTa SYBR Green FastMix 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. After thawing, mix thoroughly before using.

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

Guidelines for Fast Cycle 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 FastMix can readily amplify fragments between 400 and 500 bp; however, to take full advantage of fast cycling protocols, amplicon size should be limited to less than 150 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.

Guidelines for Fast Cycle SYBR Green 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 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 20-µL rxn.	Final Concentration
PerfeCTa SYBR Green FastMix (2X)	10.0 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)	20 µL	

Reaction Protocol

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

Initial denaturation:	95°C, variable*
PCR cycling (30-45 cycles:)	95°C, 1s
	60°C, 20 to 30 s (collect and analyze data)†
Melt Curve (dissociation stage)	Refer to instrument instructions (optional)

* Full activation of AccuFast Taq DNA polymerase occurs within 1 second at 95°C; 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 95C 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, may require as little as 1s at 95°C. Stability of RNA:DNA duplex present in first-strand product should be considered when programming the denaturation step, particularly if using oligo-dT primer and RNase H deficient reverse transcriptase. Use 30s at 95°C as a general starting point.

† Extension time is dependent upon amplicon length and minimal data collection time requirement for your qPCR instrument. 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. The use of an elevated temperature (80°C) 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 FastMix 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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