

PerfeCta® SYBR® Green FastMix®, ROX™

Cat. No.	95073-250	Size:	250 x 20-µL reactions (2 x 1.25 mL)
	95073-012		1250 x 20-µL reactions (10 x 1.25 mL)
	95073-05K		5000 x 20-µL reactions (1 x 50 mL)

Store at -20°C protected from light

Description

PerfeCta SYBR Green FastMix, 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, 7900HT, StepOne™, or StepOnePlus™ instruments. 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 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). 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 ROX provides seamless integration on the Applied Biosystems 7000, 7300, 7700, 7900HT, StepOne, or StepOnePlus. 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, 7900HT Fast, StepOne™, StepOnePlus™
PerfeCta SYBR Green FastMix, Low ROX	95074-250, 95074-012, 95074-05K	Applied Biosystems 7500, 7500 Fast, ViiA™ 7 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 CFX96™, CFX384™, Opticon™, MiniOpticon™, Chromo4™ Cepheid Smart Cycler®; Qiagen/Corbett Rotor-Gene® Eppendorf Mastercycler® ep realplex Roche Applied Science LightCycler® 480

Components

PerfeCta SYBR Green FastMix, ROX (2X):

2X reaction buffer containing optimized concentrations of MgCl₂, dNTPs (dATP, dCTP, dGTP, dTTP), AccuFast Taq DNA Polymerase, SYBR Green I dye, ROX Reference Dye, and stabilizers.

Storage and Stability

PerfeCta SYBR Green FastMix, ROX is stable for 1 year when stored in a constant temperature freezer at -20°C, protected from light. It may be stored unfrozen at +2 to +8°C for up to 6 months. After thawing, mix thoroughly by gently vortexing before using.

Repeated freezing and thawing 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, ROX 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.
- 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

Reaction Assembly

Component	Volume for 20- μ L rxn.	Final Concentration
PerfeCt _a SYBR Green FastMix, ROX (2X)	10.0 μ L	1x
Forward primer	variable	100 – 500 nM
Reverse primer	variable	100 – 500 nM
Nuclease-free water	variable	
Template	5 – 10 μ L	variable
Final Volume (μ L)	20 μ L	

Final reaction volume may vary from 10 to 50 μ L, scale all components proportionally.

After sealing each reaction, vortex gently to mix contents. Centrifuge briefly to collect components at the bottom of the reaction tube.

PCR Cycling Protocol

	Fast 2-Step Cycling	Fast 3-Step Cycling	Standard Cycling
Initial denaturation:	95°C, 30s *	95°C, 30s *	95°C, 2-3 min *
PCR cycling (30-45 cycles):	95°C, 3 to 5s	95°C, 3 to 5s	95°C, 10 to 15s
		55 to 65°C, 15s	
Collect data at end of extension step	60°C, 20 to 30s †	68 to 72°C, 10s †	60°C, 30 to 60s †
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 95°C to fully denature and fragment the template. Short double-stranded DNA template (PCR product) or single-stranded DNA template, may require as little as 1s at 95°C. 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 or primer concentration may need to be empirically determined for any given primer set and real-time instrument.

Quality Control

Kit components are free of contaminating DNase and RNase. PerfeCt_a SYBR Green FastMix, 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 $> 90\%$.

Limited Label Licenses

This product is provided under an agreement between Molecular Probes, Inc. (a wholly owned subsidiary of Invitrogen Corporation) and Quanta Biosciences, Inc., and the manufacture, use, sale or import of this product is subject to one or more of U.S. Patent Nos. 5,436,134; 5,658,751 and corresponding international equivalents, owned by Molecular Probes. The purchase of this product conveys to the buyer the non-transferable right to use the purchased amount of the product and components of the product in research conducted by the buyer, where such research does not include testing, analysis or screening services for any third party in return for compensation on a per test basis. The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party or otherwise use this product or its components or materials made using this product or its components for Commercial Purposes. Commercial Purposes means any activity by a party for consideration and may include, but is not limited to: (1) use of the product or its components in manufacturing; (2) use of the product or its components to provide a service, information, or data; (3) use of the product or its components for therapeutic, diagnostic or prophylactic purposes; or (4) resale of the product or its components, whether or not such product or its components are resold for use in research. For information on purchasing a license to this product for purposes other than research, contact Molecular Probes, Inc., Business Development, 29851

Use of this product is covered by one or more of the following US patents and corresponding patent claims outside the US: 5,994,056 and 6,171,785. The purchase of this product includes a limited, nontransferable immunity from suit under the foregoing patent claims for using only this amount of product for the purchaser's own internal research. No right under any other patent claim and no right to perform commercial services of any kind, including without limitation reporting the results of purchaser's activities for a fee or other commercial consideration, is conveyed expressly, by implication, or by estoppel. This product is for research use only. Diagnostic uses under Roche patents require a separate license from Roche. Further information on purchasing licenses may be obtained by contacting the Director of Licensing, Applied Biosystems, 850 Lincoln Centre Drive, Foster City, California 94404, USA.

The purchase of this product includes a limited, non-transferable right to use the purchased amount of the product to perform Applied Biosystems' patented Passive Reference Method for the purchaser's own internal research. No right under any other patent claim and no right to perform commercial services of any kind, including without limitation reporting the results of purchaser's activities for a fee or other commercial consideration, is conveyed expressly, by implication, or by estoppel. This product is for research use only. For information about these rights or on obtaining additional rights, please contact outlicensing@lifetech.com or Out Licensing, Life Technologies, 5791 Van Allen Way, Carlsbad, California 92008.

The purchase of this product includes a limited, non-transferable license for all fields other than human or veterinary in vitro diagnostics under specific claims of U.S. Patent Nos. 6,174,670, 6,569,627 and 5,871,908, owned by the University of Utah Research Foundation or Evotec Biosystems GmbH and licensed to Idaho Technology, Inc. and Roche Diagnostics GmbH, to use only the enclosed amount of product according to the specified protocols. No right is conveyed, expressly, by implication, or by estoppel, to use any instrument or system under any claim of U.S. Patent Nos. 6,174,670, 6,569,627 and 5,871,908, other than for the amount of product contained herein.

Licensed to Quanta Biosciences, under U.S. Patent Nos. 5,338,671, 5,587,287, and foreign equivalents for use in research only.

PerfeCt_a, FastMix, AccuFast and AccuStart are trademarks of Quanta Biosciences Inc. TaqMan is a registered trademark of Roche Molecular Systems, Inc. LightCycler is a registered Trademark of Roche. Applied Biosystems, StepOne, StepOnePlus, ViiA, and ROX are trademarks Life Technologies Corporation. Stratagene, MX3000P, MX3005P and MX4000 are trademarks of Agilent Technologies, Inc. Mastercycler is a trademark of Eppendorf. Rotor-Gene is a registered trademark of Qiagen GmbH. SmartCycler is a trademark of Cepheid. CFX96, CFX384, iCycler iQ, iQ5, MyiQ, Opticon, MiniOpticon and Chromo4 are trademarks of Bio-Rad Laboratories. SYBR is a registered Trademark of Molecular Probes, Inc.

©2007 Quanta BioSciences, Inc. All rights reserved.

For research use only. Not intended for any animal or human therapeutic or diagnostic use.