

PerfeCta™ SYBR® Green SuperMix, UNG, ROX

Cat No.	95069-100	Size:	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)

Store at -20°C protected from light

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 MgCl₂, 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- μ L rxn.	Final Concentration
PerfeCta SYBR Green SuperMix, UNG, ROX (2X)	25 μ L	1x
Forward primer	variable	100 – 500 nM
Reverse primer	variable	100 – 500 nM
Nuclease-free water	variable	
Template	<u>5 – 10 μL</u>	variable
Final Volume (μ L)	50 μ L	

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

Reaction Protocol

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

UNG incubation	45°C, 5 min
Initial denaturation:	95°C, 2 to 3 min
PCR cycling (30-45 cycles):	95°C, 10 to 15 s
	55 – 65°C, 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 95°C. 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 72°C 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 (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 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 $> 90\%$.

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

This product is sold under licensing arrangements between Quanta BioSciences, Inc. and Invitrogen Corporation. This product or its use is covered by at least one claim of U.S. Pat. Nos. 5,035,996; 5,683,896; 5,945,313; 6,287,823; or 6,518,026, owned by Invitrogen Corporation. 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 (whether the buyer is an academic or for-profit entity). The buyer cannot sell or otherwise transfer (a) this product, (b) its components, or (c) materials made by the employment of this product or its components to a third party or otherwise use this product or its components or materials made by the employment of this product or its components for Commercial Purposes. Commercial Purposes means any activity for which a party receives or is due to receive 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. The buyer cannot use this product or its components or materials made using this product or its components for therapeutic, diagnostic or prophylactic purposes. Further information on purchasing licenses under the above patents may be obtained by contacting the Licensing Department, Invitrogen Corporation, 1600 Faraday Avenue, Carlsbad, CA 92008. Email: outlicensing@invitrogen.com.

Use of this product is covered by one or more of the following US patents and corresponding patent claims outside the US: 5,079,352, 5,789,224, 5,618,711, 6,127,155, 5,677,152, 5,773,258, 5,407,800, 5,322,770, 5,310,652, 5,994,056, 6,171,785, and claims outside the US corresponding to US Patent No. 4,889,818. The purchase of this product includes a limited, non-transferable 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 (such as apparatus or system claims in US Patent No. 6,814,934) 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.

Licensed to Quanta BioSciences, under U.S. Patent Nos. 5,338,671, 5,587,287, and foreign equivalents.

PerfeCta 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 and ROX are trademarks of the Applied Biosystems Corporation. Stratagene, MX3000P, MX3005P and MX4000 are trademarks of Stratagene Corporation. Mastercycler is a trademark of Eppendorf. Rotor-Gene is a trademark of Corbett Life Science. SmartCycler is a trademark of Cepheid. 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.