

Product Information

KiCqStart™ SYBR® Green qPCR ReadyMix™, Low ROX™

Catalog Number **KCQS01**

Storage Temperature -20 °C

Technical Bulletin

Product Description

KiCqStart SYBR Green qPCR ReadyMix, Low ROX is a 2X concentrated, ready-to-use reaction cocktail that contains all components, except primers and template, for real-time quantitative PCR (qPCR) on Applied Biosystems 7500, 7500 Fast, ViiA™ 7, or Stratagene MX series of real-time PCR systems. This unique combination of proprietary buffer, stabilizers, and KiCqStart Taq DNA polymerase delivers 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. KiCqStart 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 the enzyme is instantaneous at 95 °C. Replication of fragments up to 200 bp is complete in less than 20 sec at 60 °C

Reagents

2X reaction buffer containing optimized concentrations of MgCl₂, dNTPs, (dATP, dCTP, dGTP, dTTP), KiCqStart Taq DNA Polymerase, SYBR Green dye, ROX Reference Dye (for 580-585 nm excitation), and stabilizers.

KiCqStart SYBR Green	KCQS01-250RXN
qPCR ReadyMix, Low ROX	KCQS01-1250RXN
	KCQS01-5000RXN

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

Stable for 1 year when stored -20 °C and protected from light. It may be stored at 2-8 °C for up to 6 months. After thawing, mix thoroughly by gentle vortexing before using.

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

Usage Guidelines

- 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. KiCqStart SYBR Green qPCR ReadyMix, Low 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 separate reaction tubes. Add the DNA template to each reaction as the final step. Dilution of template samples to allow at least a 5-10 µL addition 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

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. KiCqStart SYBR Green qPCR ReadyMix, 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.sigmaaldrich.com to find the optimal kit for your instrument platform.

Instrument Applications

Real-time PCR Instrument Name	KiCqStart SYBR Green qPCR ReadyMix	KiCqStart SYBR Green qPCR ReadyMix Low ROX	KiCqStart SYBR Green qPCR ReadyMix with ROX	KiCqStart SYBR Green qPCR ReadyMix iQ
	KCQS00	KCQS01	KCQS02	KCQS03
Applied Biosystems 5700			•	
Applied Biosystems 7000			•	
Applied Biosystems 7300			•	
Applied Biosystems 7500		•		
Applied Biosystems 7500 Fast		•		
Applied Biosystems 7700			•	
Applied Biosystems 7900			•	
Applied Biosystems 7900 HT Fast			•	
Applied Biosystems 7900HT			•	
Applied Biosystems StepOnePlus™			•	
Applied Biosystems StepOne™			•	
Applied Biosystems ViiA 7		•		
Bio-Rad CFX384™	•			
Bio-Rad CFX96™	•			
BioRad iCycler iQ™				•
BioRad iQ™5				•
Bio-Rad MiniOpticon™	•			
BioRad MyiQ™				•
Bio-Rad/MJ Chromo4™	•			
Bio-Rad/MJ Opticon 2	•			
Bio-Rad/MJ Opticon®	•			
Cepheid SmartCycler®	•			
Eppendorf Mastercycler® ep realplex	•			
Eppendorf Mastercycler® ep realplex2 s	•			
Illumina Eco qPCR	•			
Qiagen/Corbett Rotor-Gene® 3000	•			
Qiagen/Corbett Rotor-Gene® 6000	•			
Qiagen/Corbett Rotor-Gene® Q	•			
Roche LightCycler™ 480	•			
Stratagene Mx3000P®		•		
Stratagene Mx3005P™		•		
Stratagene Mx4000™		•		

Reaction Assembly

Reagent	Volume for 20 μ L reaction	Final Concentration
KiCqStart SYBR Green qPCR ReadyMix, Low ROX (2X)	10.0 μ L	1X
Forward Primer	variable	100 – 500 nM (start with 300 nM)
Reverse Primer	variable	100 – 500 nM (start with 300 nM)
Nuclease-free water	variable	Add to q.s. to 20 μ L
Template	<u>at least 5 μL</u>	variable
Final Volume (μ L)	20 μ L	

Final reaction volume may vary from 10-50 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, 30 sec *	95 °C, 30 sec *	95 °C, 2-3 min *
PCR cycling (30-45 cycles):	95 °C, 3-5 sec	95 °C, 3-5 sec	95 °C, 10-15 sec
		55-65 °C, 15 sec	
Collect data at end of extension step	60 °C, 20-30 sec †	68-72 °C, 10 sec †	60 °C, 30-60 sec †
Melt Curve (dissociation stage)	Refer to instrument instructions (optional)		

* Full activation of KiCqStart 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 5-10 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 1 sec 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. KiCqStart SYBR Green qPCR ReadyMix, 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 $> 90\%$.

SYBR is a registered trademark of Molecular Probes, Inc., ReadyMix is a trademark of Sigma-Aldrich Co. LLC, KiCqStart is a trademark of Quanta BioSciences, 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.

Limited Label Licenses

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.

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

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.

Applicable only to products containing Passive Reference 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.

SC,PHC 01/12-1

©2012 Sigma-Aldrich Co. LLC. All rights reserved. SIGMA-ALDRICH is a trademark of Sigma-Aldrich Co. LLC, registered in the US and other countries. Sigma brand products are sold through Sigma-Aldrich, Inc. Purchaser must determine the suitability of the product(s) for their particular use. Additional terms and conditions may apply. Please see product information on the Sigma-Aldrich website at www.sigmaaldrich.com and/or on the reverse side of the invoice or packing slip.