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Product Information

KiCqStart™ SYBR® Green qPCR ReadyMix™, iQ

Catalog Number **KCQS03**
Storage Temperature –20 °C

Technical Bulletin

Product Description

KiCqStart SYBR Green qPCR ReadyMix, iQ is a 2X concentrated, ready-to-use reaction cocktail that contains all components, except primers and template, for real-time quantitative PCR (qPCR) on Bio-Rad iCycler iQ® 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 $MgCl_2$, dNTPs, (dATP, dCTP, dGTP, dTTP), KicqStart Taq DNA Polymerase, SYBR Green dye, 20 nM fluorescein, and stabilizers.

KiCqStart SYBR Green qPCR ReadyMix, iQ	KCQS03-250RXN KCQS03-1250RXN KCQS03-5000RXN

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, 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, iQ 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 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, iQ contains fluorescein for experimental plate well factor collection on iCycler iQ real-time detection systems or the MyiQ detection system. 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 µL reaction	Final Concentration
KiCqStart SYBR Green qPCR ReadyMix, iQ (2X)	10.0 µL	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 µL
Template	at least 5 µL	variable
Final Volume (µL)	20 µL	

Final reaction volume may vary from 10-50 µL , 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.

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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 ºC, 15 sec	
Collect data at end of extension step	60 °C, 20-30 sec †	68 °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 ºC; 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 ºC 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 ºC. Use 30 sec at 95 ºC 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. PerfeCTa SYBR Green ReadyMix for iQ 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

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