SimplyGreen qPCR Master Mix, No Rox

Cat. No.: SQ103-0100 Size: 100 rxns (for 20 µl/ rxns) / 200 rxns (for 10 µl/ rxns)
Cat. No.: SQ103-0020 Size: 20 rxns (for 20 µl/ rxns) / 40 rxns (for 10 µl/ rxns)
Storage: Stable for up to 1 year at -20°C

Description

A 2X concentrated mix of *Taq* polymerase, dNTPs, $MgCl_2$, fluorescent dye (detection), and proprietary buffer components, the SimplyGreen qPCR Master Mix, No Rox provides a convenient, reliable and robust set-up for performing quantitative real-time analysis of DNA samples. Designed specifically for the aforementioned niche of application, the components of SimplyGreen qPCR Master Mix, No Rox promise topnotch performance with respect to sensitivity, signal-to-noise ratio and elimination of primer dimers. Furthermore, the proprietary chemical modification of the DNA polymerase included in this mastermix allows for hot-start PCR, conferring a significant reduction in non-specific PCR amplification that is otherwise of a common occurrence with regular Taq polymerases.
Based on the fact that the qPCR instruments can vary from user to user, we suggest that the SimplyGreen qPCR Master Mix, No Rox is compatible with qPCR instrument: BioRad® CFX96, CFX384, Chromo4™, CFX Connect™, Opticon 2, MiniOpticon™; Roche LightCycler® (2.0, 1.5, 480, 1536,Nano); MJ Research Opticon™, Opticon™2, Chromo® 4; Corbett Rotor-gene® (3000,6200, 62H0, 6500, 65H0, 6600).

Kit contents

Contents	SQ103-0100	SQ103-0020
SimplyGreen qPCR Master Mix, No Rox	1000 µl	200 µl

Required Materials

➢ Real-time PCR tubes ➢ Real-time PCR instrument ➢ RNase-Free H_2O

Real-time PCR Instrument

Product Name	Real-time PCR Instrument
SimplyGreen qPCR Master Mix, No Rox	BioRad® CFX96, CFX384, Chromo4™, CFX Connect™, Opticon 2, MiniOpticon™, Roche LightCycler® (480, 1536, Nano) MJ Research Opticon™, Opticon™ 2, Chromo® 4, Enigma® ML Eppendorf® Realplex 4, BioGene SynChron™ Corbett Rotor-gene® (3000, 6200, 62H0, 6500, 65H0, 6600) Eppendorf Mastercycler® realplex (s, 4 , 4s), Pro (S, 384), Nexus (gradient, eco, flat), Cepheid SmartCycler®, GeneXpert Idaho LightScanner® (24, 32), RapidCycler®2, R.A.P.I.D (LT, LT Food), RAZOR EX, JBAIDS, Qiagen Rotor-Gene™ (Q, 6000), Takara Dice™ Thermo Scientific PikoReal, DNA-Technology DT96, DTlite, DT-322 Bioer LineGene (3310/3320, K FQD-48A, I, II, 9620, 9640, 9660, 9680) Bioneer Exicycler™

Application

➢ Gene Expression (mRNA) Analysis ➢ Genetic Variation Analysis
➢ microRNA & Noncoding RNA Analysis

Storage Conditions

Upon arrival, the SimplyGreen qPCR Master Mix, No Rox should be stored at -20°C and protected from light. After each experiment, the leftover thawed mix can be stored at 4°C if it is to be used within the next 3 months. Avoid repeated freeze-thaw cycles to retain maximum performance. The SimplyGreen qPCR Master Mix, No Rox is stable for 1 year from the date of shipping when stored and handled properly.

Protocol

1. Thaw the SimplyGreen qPCR Master Mix, No Rox, template DNA, primers and nuclease-free water on ice. Mix each solution well.
2. Set up the following reaction mixture (10 µl or 20 µl reaction volume):

Components	10 µl Reaction	20 µl Reaction	Final Concentration
SimplyGreen qPCR Master Mix, No Rox	5 µl	10 µl	1X
Forward Primer (10 µM)	0.3 µl	0.6 µl	300 nM
Reverse Primer (10 µM)	0.3 µl	0.6 µl	300 nM
Template DNA	Variable	Variable	≤500 ng/reaction
Nuclease-free H_2O	to 10 µl	to 20 µl	

3. Perform qPCR reactions using the following cycling program:

Step	Temperuture	Duration (Standard Mode)	Duration (Fast Mode)	Cycle(s)
Enzyme Activation	95°C	10 minutes	20 seconds	1
Denaturation	95°C	15 seconds	3 seconds	30-35
Annealing/ Extension	60°C	60 seconds	30 seconds	
Melting Curve	Refer to specific guidelines for instrument used			

Note:
Optimal conditions for amplification will vary depending on the primers and thermal cycler used. It may be necessary to optimize the system for individual primers, template, and thermal cycler.

Recommendations for Optimal Results:
➢ Aliquot reagents to avoid contamination and repeated freeze-thaw cycles.
➢ Ideally, start the PCR as soon as the reaction mixture is prepared. If not, then make sure that the reaction mixture is kept chilled till starting up the PCR.
➢ For gDNA amplification, use 2 minutes enzyme activation time instead of 30 seconds.
➢ 10 - 15 seconds annealing/extension time is preferred unless restricted by the software.
➢ SimplyGreen qPCR Master Mix, no Rox components are light sensitive and therefore, avoid prolonged direct exposure to light.

Troubleshooting

Refer to the table below to troubleshoot problems that you may encounter when quantify of nucleic acid targets with the kit.

Trouble	Cause	Solution
Poor Signal or No Signal	Inhibitor Present	1. Perform a dilution series of the PCR template to determine whether the effect of the inhibitory agent can be reduced. 2. Take extra care with the nucleic acid extraction steps to minimize carryover of PCR inhibitors.
	Degraded Template Materiall	1. Do not store diluted template in water or at low concentrations. 2. Check the integrity of template material by automated or manual gel electrophoresis.
Signal in Negative Control	Contamination of Reaction Components with Target Sequence	1. To minimize the possibility of contamination of PCR components by PCR product or other template, designate a work area exclusively for PCR assay setup. 2. Use a solution of 10% bleach instead of ethanol to prepare the workstation area for PCR assay setup. Ethanol will only induce precipitation of DNA in your work area, while the 10% bleach solution will hydrolyze, as well as dissolve, any residual DNA.
Poor Reproducibility Across Replicate Samples	Inhibitor Present	1. Perform a dilution series of the PCR template to determine whether the effect of the inhibitory agent can be reduced. 2. Take extra care with the nucleic acid extraction steps to minimize carryover of PCR inhibitors.
	Primer Design	1. Verify primers design at different annealing temperatures.
Low or High Reaction Efficiency	Primer- Dimer	1. Reduce primer concentration. 2. Evaluate primer sequences for complementarity and secondary structure. Redesign primers if necessary. 3. Perform melt-curve analysis to determine if primer- dimers are present.
	Insufficient Optimization	1. Use a thermal gradient to identify the optimal thermal cycling conditions for a specific primer set.

Caution

1. Shake gently before use to avoid foaming and low-speed centrifugation.
2. Reduce the exposure time.
3. This product is not available for hybridization probe method.
4. During operation, always wear a lab coat, disposable gloves, and protective equipment.
5. Research Use Only. Not intended for any animal or human therapeutic or diagnostic uses.

Related Ordering Information

Cat. No.	Description	Size
SN017-0100	Total RNA Isolation Kit (Blood Cultured Cell Fungus)	100 Reactions
SN020-0100	Total RNA Isolation Kit (Plant)	100 Reactions
SN016-0100	Virus Nucleic Acid Isolation Kit	100 Reactions
SM303-0050	GScript RTase	50 Reactions
SM305-0050	GScript First-Strand Synthesis Kit	50 Reactions