EvaGreen qPCR System-ROX I

Cat No. QP005-0100 Size: 100 rxns (for 20 µl/ rxns) / 200 rxns (for 10 µl/ rxns)
Cat No. QP005-0020 Size: 20 rxns (for 20 µl/ rxns) / 40 rxns (for 10 µl/ rxns)
Storage: Stable for up to 1 year at -20°C

Description

The EvaGreen qPCR System-ROX I provides a convenient, simple, rapid, high sensitivity, specificity, stability, and robust set-up for performing quantitative real-time analysis of DNA samples. EvaGreen qPCR System-ROX I with the proprietary concentration with the of HotStart Taq DNA Polymerase, dNTPs, $MgCl_2$, fluorescent dye (detection), reference dye and proprietary buffer components, the EvaGreen qPCR System-ROX I provides a convenient and reliable set-up for performing quantitative real-time analysis of DNA samples.
Designed specifically for this niche of application, the components of EvaGreen qPCR System-ROX I promise top performance with respect to sensitivity, signal-to-noise ratio and elimination of primer dimers. Furthermore, GeneDirex's most efficient HotStart DNA Polymerase included in this SuperMix allows for ultra fast PCR, conferring a significant reduction to the overall qPCR quantification and detection time, thus streamlining the experiment through cost and labor saving.
In light of the fact that the qPCR instruments can vary from user to user, GeneDireX offers the EvaGreen qPCR System-ROX I in a range of formulations, each of which has been carefully optimized to confer the best performance according to the make and model of a qPCR machine.
The EvaGreen qPCR System-ROX I is supplied at the 2X concentration to allow approximately 50% of the final reaction volume to be used for the addition of primer, template solutions, and RNase– free H_2O. The Reagent is provided with the sufficient amplification reactions of 10 or 20 µl each.

Kit contents

Contents	QP005-0100	QP005-0020
EvaGreen qPCR System-ROX I	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
EvaGreen qPCR System-ROX I	ABI® 7000, 7300, 7700, 7900, 7900HT StepOnePlus™; StepOne™; OpenArray PRISM™ Sequencing Detection Series

Application

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

Storage Conditions

Upon arrival, the EvaGreen qPCR System-ROX I 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 EvaGreen qPCR System-ROX I is stable for 1 year from the date of shipping when stored and handled properly.

Protocol

1. Thaw the EvaGreen qPCR System-ROX I , 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
EvaGreen qPCR System-ROX I	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:

Initial Denaturation	95°C	30 secs	1
Denaturation	95°C	3 - 5 secs	35 - 40
Annealing / Extension	55°C - 60°C	10 - 30 secs	
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 the reagent to avoid contamination and repeated freeze-thaw cycles.
➢ Note that the EvaGreen qPCR System-ROX I components are light sensitive and therefore, avoid prolonged exposure to direct light.
➢ 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 secs annealing/extension time is preferred unless restricted by the software.
➢ Aliquot reagents to avoid contamination and repeated freeze-thaw cycles.
➢ EvaGreen qPCR System- ROX I components are light sensitive and therefore, avoid prolonged direct exposure to light.
➢ Perform PCR as soon as the reaction mixture is prepared; otherwise keep everything chilled or frozen meanwhile.

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 Material	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.
	Inadequate Thermal Cycling Conditions	1. Try using a minimum extension time of 30 sec for genomic DNA and 15 sec for cDNA.

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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
NA017-0100	Total RNA Isolation Kit (Blood Cultured Cell Fungus)	100 Reactions
NA020-0100	Total RNA Isolation Kit (Plant)	100 Reactions
NA016-0100	Virus Nucleic Acid Isolation Kit	100 Reactions
MB303-0050	GScript RTase	50 Reactions
MB305-0050	GScript First-Strand Synthesis Kit	50 Reactions