

BIO BASIC Worldwide



For more information on pricing, complete product line or to locate a Point of Sales near you, please visit our web site or contact one of our Customer Service Representatives.

Email	✉	order@biobasic.com
Phone	☎	1 (905) 474-4493
Toll Free	☎	1 (800) 313-7224
Fax	☎	1 (905) 474-5794



Green-2-Go qPCR Mastermix

QPCR001/QPCR002/
QPCR004
QF 24 TV4
CV2 2020

For Research Use Only

Green-2-Go qPCR Mastermix

Code: QPCR001-R (ROX)
QPCR002-L (Low ROX)
QPCR004-S (S)

Introduction.....	1
Description.....	1
Storage.....	2
Application.....	2
Reaction Setup.....	2
Recommendations for Optimal Results.....	3
Troubleshooting Guide.....	3
Reference Dye.....	4
ROX Reference Dye Protocol.....	4
Selection Guide.....	4

Introduction:

This series of kit is designed for quantitative real-time analysis of DNA samples by measuring the increase in fluorescence caused by the binding of Green-2-Go dye to double-stranded (ds) DNA. It is available in a ready-to-use format, compact with a wide range of real-time cyclers.

Description:

Green-2-Go qPCR Mastermix is a convenient premix of all the components, 2X mix of dNTPs, Hotstart Taq polymerase, MgCl₂, fluorescent detection dye, reference dye, and proprietary buffer components. The components of Green-2-Go qPCR Mastermix have been developed for superb performance in sensitivity, signal-to-noise ratio, and complete elimination of primer dimers. The chemically modified Hotstart Taq polymerase included in our mastermix significantly reduces nonspecific PCR amplification observed with regular Taq polymerase.

Due to variations in qPCR instruments, we offer different Green-2-Go qPCR Mastermix formulations optimized for different machines. Please use the following table as a guideline for the selection of qPCR Mastermix appropriate for your particular instrument model.

Producing Company	Name of Machine	CAT number
Takara takara-bio.com	Thermal Cycler Dice™ Real Time System	QPCR004-S
Illumina illumina.com	Eco Real-Time PCR System	QPCR002-L
Analytikjena analytik-jena.de	qTower	QPCR002-L
	qTower 2.0	QPCR002-L
	qTower 2.2	QPCR002-L
	qTower 3	QPCR002-L
Biometra biometra.de	Optical Thermocycler	QPCR001-R
Fluidigm	BioMark™ HD System	QPCR001-R
DNA-Technology dna-technology.com	DT-96 (same as DT prime)	QPCR004-S
	DT-96 (5 filters)	QPCR004-S
	DTlite	QPCR004-S
	DT-322	QPCR004-S
Bioer Technology bioer.com.cn/bioer/bioer_en	LineGene3310/3320 Real-Time PCR Detection System	QPCR004-S
	LineGene K FQD-48A(M2)	QPCR004-S
	LineGene K FQD-48A(A4)	QPCR004-S
	Line Gene I	QPCR004-S
	Line Gene II	QPCR004-S
	Line Gene 9620	QPCR004-S
	Line Gene 9640	QPCR004-S
	Line Gene 9660	QPCR004-S
Bioneer bioneer.com/	Line Gene 9680	QPCR004-S
	Exicycler™	QPCR004-S
Corbett Corbettlifescience.com	Rotor Gene 3000	QPCR004-S
	Rotor Gene 6200	QPCR004-S
	Rotor Gene 62H0	QPCR004-S
	Rotor Gene 6500	QPCR004-S
	Rotor Gene 65H0	QPCR004-S
Thermo Scientific thermoscientific.com/pikoreal	Rotor Gene 6600	QPCR004-S
	PikoReal	QPCR004-S
	SmartChip System	QPCR001-R
Wafergene	TL998 System	QPCR001-R
Abbott Molecular abbottmolecular.com	m2000RT	QPCR002-L
Funglyn http://www.funglyn.com	FTC-3000/ FTC-3000p	QPCR004-S
ESCO http://www.escoglobal.com	Swift™ Spectrum 96 SPT96-4	QPCR004-S
	Swift™ Spectrum 96 SPT96-8	QPCR004-S
	Swift™ Spectrum 48 SPT48	QPCR004-S
Techne http://techne.com	Quantica	QPCR004-S
	Prime Pro 48	QPCR004-S
	Prime Q	QPCR004-S

Producing Company	Name of Machine	CAT number
Bio-Rad Laboratories bio-rad.com	CFX96 Touch™ Real-Time PCR Detection System	QPCR004-S
	CFX384 Touch™ Real-Time PCR Detection System	QPCR004-S
	Chromo4™ Four-Color Real-Time Detector	QPCR004-S
	CFX Connect™ Real-Time PCR Detection System	QPCR004-S
	Opticon 2 - Continuous Fluorescence Detection System	QPCR004-S
Cepheid: cepheid.com	MiniOpticon™ Real-Time PCR Detection System	QPCR004-S
	SmartCycler®	QPCR004-S
Eppendorf: eppendorf.com	GeneXpert	QPCR004-S
	Mastercycler® ep realplex, Real-Time Thermal Cycler	QPCR004-S
	Mastercycler® ep realplex s, Real-Time Thermal Cycler	QPCR004-S
	Mastercycler® ep realplex 4, Real-Time Thermal Cycler	QPCR004-S
	Mastercycler® ep realplex 4s, Real-Time Thermal Cycler	QPCR004-S
	Mastercycler Pro	QPCR004-S
	Mastercycler Pro S	QPCR004-S
	Mastercycler Pro 384	QPCR004-S
	Mastercycler Nexus	QPCR004-S
	Mastercycler Nexus gradient	QPCR004-S
Enigma Diagnostics: enigmadiagnostics.com	Mastercycler Nexus eco	QPCR004-S
	Mastercycler Nexus flat	QPCR004-S
Idaho Technologies: idahotech.com	Enigma® ML	QPCR004-S
	LightScanner® 24 System	QPCR004-S
	LightScanner® 32 System	QPCR004-S
	RapidCycler® 2 System	QPCR004-S
	R.A.P.I.D. System	QPCR004-S
	RAZOR EX Instrument	QPCR004-S
	R.A.P.I.D. LT System	QPCR004-S
	R.A.P.I.D. LT Food Security System	QPCR004-S
Roche Diagnostics Ltd: roche-applied-science.com	JBAIDS System	QPCR004-S
	LightCycler® 2.0 Instrument	QPCR004-S
	LightCycler® 1.5 Instrument	QPCR004-S
	LightCycler® 96 system	QPCR004-S
	LightCycler® 480 System (system I)	QPCR004-S
	LightCycler® 480 System (System II)	QPCR004-S
Agilent http://www.agilent.com/home	LightCycler® 1536 System	QPCR004-S
	LightCycler® Nano System	QPCR004-S
	Mx3000P® qPCR System	QPRC002-L
	Mx3005P® qPCR System	QPRC002-L
Qiagen qiagen.com	Mx4000® qPCR System	QPRC002-L
	AriaMx Realtime PCR System	QPRC002-L
Qiagen qiagen.com	Rotor-Gene™ Q - Pure Detection	QPCR004-S
	Rotor-Gene™ 6000 (see Corbett Rotor-gene series below)	QPCR004-S

Product Code	Description	qPCR Instruments	Quantity (500 Rxn. X 20ul)
QPCR001-R (2X)	Green-2-Go qPCR -ROX	ABI® 7000, 7300, 7700, 7900, 7900HT; StepOnePlus™; StepOne™; OpenArray; PRISM™ Sequencing Detection Series	4 x 1.25 ml
QPCR002-L (2X)	Green-2-Go qPCR Low ROX	ABI® 7500; Viia™; QuantStudio; Illumina Eco; Stratagene® Mx3000, Mx3005, Mx4000	4 x 1.25 ml
QPCR004-S (2X)	Green-2-Go qPCR-S	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)	4 x 1.25 ml

Storage:

Transportation at frozen temperature. Green-2-Go qPCR Mastermix 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 kit is stable for 1 year under these conditions.

Application:

1. Gene expression analysis.
2. Microarray validation.
3. Viral load determination.

Reaction Setup:

1. Thaw Green-2-Go qPCR Mastermix, template DNA, primers and RNase-free water on ice. Mix each solution well. Prepare a reaction Mastermix using the following:

Components	10 µl Reaction	20 µl Reaction	Final Concentration
Green-2-Go Mastermix	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
RNase-Free ddH2O	Variable	Variable	
Template DNA	Variable	Variable	less than or equal to 500 ng/reaction
Total Volume	10 µl	20 µl	

2. Perform qPCR reactions using the following cycling program.

Step	Temperature	Duration(Standard)	Duration (Fast)	Cycles (S)
Enzyme activation	95°C	5 min	20 sec	1
Duration	95°C	15 sec	3 sec	40
Anneal/extend	60°C	60 sec	30 sec	
Melting curve	Refer to specific guidelines for instrument used.			

Recommendations for Optimal Results:

1. Aliquot reagents to avoid contamination and to avoid repeated freeze-thaw cycles.
2. Green-2-Go qPCR Mastermix components are light sensitive; avoid exposure to light.
3. Start PCR as soon as the reaction mixture is prepared and always keep the reaction mixture chilled in an ice box prior to PCR reactions.

Troubleshooting Guide:

No fluorescence signal at all	
Possible Cause	Resolution
Error in cyler setup.	Check that instrument settings correspond with the experiment.
Missing components (e.g. primers, probe or template).	Check the assembly of the reaction.
Probe is not labelled very well.	Re-label probe.
Missing essential step in the cyler protocol.	Check the cyler protocol.
Sample configured as empty.	Check the plate configuration.
Late increase in fluorescence signal	
Possible Cause	Resolution
Insufficient starting template.	Check the calculation of template stock concentration; Increase template amount if possible.
Annealing temperature too high.	Use gradient to optimize annealing temperature; Decrease annealing temperature in 2°C decrements if a gradient feature is not available.
Probe is not labelled very well.	Re-label probe.
Insufficient extension time for the amplicon size.	Increase extension time.
Primer or probe concentration too low.	Increase primer concentration (to max 900 nM each). 250 nM probe concentration is usually sufficient.
PCR protocol not optimal.	Make sure the recommended PCR protocol is used. If necessary, optimize using the recommended protocol as a starting point.
Normal fluorescence signal, but low efficiency	
Possible Cause	Resolution
Pipetting error.	Check the assembly of the reactions.
Primer-dimers from previous run contaminating the reaction.	Perform UNG treatment prior to PCR cycling.
Primer and probe design not optimal or very low template concentration.	Re-check primer and probe design and template stock concentration.
Probe is not labelled very well.	Re-label probe.
Inhibitors from the sample affecting reaction.	Repurify DNA.
Low initial template concentration.	Increase template amount.
Non-linear correlation between C(t) and log of template amount in standard curve	
Possible Cause	Resolution
Template dilution inaccurate.	Remake dilution series and make sure the samples are well mixed.
Template amount too high.	Reduce the template amount; Increase reaction volume.
Template amount too low.	Increase template amount.
Primer-dimers co-amplified.	Redesign primers.

Reference Dye:

Pipetting errors and instrument limitations can become inherently detrimental when viewed in the context of experiments like real-time quantitative PCR or RT-PCR. Therefore, in order to proactively compensate for such inadvertent yet common sources of errors, a reference dye can be very helpful. Inert by nature, a reference dye does not undergo any fluorescence change during experiments like real-time quantitative PCR or RT-PCR.

Therefore, the addition of a reference dye helps normalize the fluorescent reporter signal in the aforementioned experiments by allowing the sware/instrument to adjust for minute differences or well-to-well inconsistencies. In this way, a reference dye enables minimal standard error (with respect to replicates in each experiment) and improves the overall performance of each experiment.

Different companies optimized their instruments with different reference dyes (ROX, Fluorescein, etc). BBI offers its Green-2-Go qPCR MasterMix in a comprehensive array of options where each MasterMix is preconfigured with a reference dye specific for a particular instrument. This way, the customers get to choose what works best for their instrument while having the advantage of using a reference dye.

ROX Reference Dye Protocol:

Product Components	Quantity
Green-2-Go qPCR Mastermix	500 rxn (4 x 1.25 ml)
ROX Reference Dye	50 µl

The recommended amount of ROX Reference Dye to be added into the MasterMix may vary depending on the qPCR machine type:

- No ROX equipment: Not needed.
- Low ROX equipment: 1 µl/1.25 ml MasterMix.
- High ROX equipment: 11 µl/1.25 ml MasterMix.

Selection Guide:

Please refer and adhere to the guide shown below and in the following pages to select the Master-Mix that will best serve your needs:

Producing Company	Name of Machine	CAT number
Applied Biosystems: appliedbiosystems.com	StepOne™ Real-Time PCR System	QPRC001-R
	StepOnePlus™ Real-Time PCR System	QPRC001-R
	7500 Real-Time PCR System	QPRC002-L
	7500 Fast Real-Time PCR System	QPRC002-L
	7500 Fast Dx Real-Time PCR Instrument	QPRC002-L
	7500 Real-Time PCR System for Human Identification	QPRC002-L
	7300 Real-Time PCR System	QPRC001-R
	Vii™ 7 Real-Time PCR System	QPRC002-L
	7900HT Fast Real-Time PCR System	QPRC001-R
	OpenArray® Real-Time PCR Platform	QPRC001-R
	QuantStudio™ 12K Flex system	QPRC002-L
	PRISM® 7000 Sequencing Detection System	QPRC001-R
	PRISM® 7700 Sequencing Detection System	QPRC001-R
	PRISM® 7900 Sequencing Detection System	QPRC001-R
	Gene Amp 5700	QPRC001-R
BioGene: biogene.com	SynChron™	QPCR004-S
	InSyte™	QPCR002-L