

Product information

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Green-2-Go 1-Step TaqProbe RT-qPCR Mastermix-iCycler

Catalog #: QPCR008-iC
Size: 100 Rxns
Storage: -20°C

Components

Components	
TaqProbe qPCR MasterMix	1.25 ml
RT-qPCR Enzyme Mix	40 µl
ROX Reference Dye	15 µl
Nuclease-free H ₂ O	1 ml
Size	100rxns

Description:

A complete qPCR system, the Green-2-Go 1-Step TaqProbe RT-qPCR Mastermix Kit provides convenience using the RT-qPCR Enzyme Mix and TaqProbe qPCR MasterMix for both highly sensitive and specific reverse transcription, and Taq probe-based real-time qPCR amplification in a single reaction tube. Our proprietary RT-qPCR Enzyme Mix contains stabilizers and enhancers that optimize the two reactions in a “single step”. This offers the end user flexibility with RNA templates and primer selection, an efficient and easy-to-use set up, and a reliable alternative to conventional “two-step” sequential RT-qPCR. The MasterMix is suitable for SNP genotyping assays, gene expression analysis, microarray validation, and high throughput screening applications. ROX Reference Dye is provided separate from the MasterMix, making this kit universally compatible with most qPCR instruments.

Protocol:

ROX Reference Dye is provided separate from the MasterMix, making this kit universally compatible with most qPCR instruments.

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.

Reactions should be assembled in an RNase-free environment. The use of “clean” pipettors designated for PCR and aerosol-resistant barrier tips are recommended.

1. Prepare the following reaction mixture in a qPCR tube on ice:

Components	Reaction Volume	Final Concentration
Total RNA or Poly(A) + mRNA ¹	Variable	1 pg - 2 µg/rxn
TaqProbe qPCR MasterMix	10 µl	1X
RT-qPCR Enzyme Mix	0.4 µl	1X
Forward Primer	Variable	100 - 500 nM
Reverse Primer	Variable	100 - 500 nM
TaqProbe	Variable	100 - 500 nM
Nuclease-free H ₂ O	to 20 µl	-

¹Amplicon should be < 150 bp in size

2. Gently mix the reaction components and briefly centrifuge.

3. Thermocycling conditions for standard qPCR:

Step	Temperature	Duration	Cycle(s)
cDNA synthesis	50°C	15 mins	1
Pre-Denaturation	95°C	3 mins	1
Denaturation	95°C	15 secs	40
Annealing	60°C	60 secs	

General Notes:

- Both poly(A) + mRNA and total RNA can be used for first-strand cDNA synthesis, but poly(A) + mRNA may give higher yields and improved purity of final products.
- RNA samples must be free of genomic DNA contamination.
- For longer transcripts >9 kb, yields can be increased by lengthening the cDNA synthesis incubation at 50°C to 30-60 minutes.



PRODUCTS ARE INTENDED FOR BASIC SCIENTIFIC RESEARCH ONLY.
 NOT INTENDED FOR HUMAN OR ANIMAL USE.