Product Information

Green-2-Go 1-Step RT-qPCR Mastermix-No Dye

Product information for QPCR007-NODYE:

Kit Component:

Components	100 Reactions (20 per reaction)
Green-2-Go qPCR Mastermix- NODYE	1.25 ml
qRT-PCR Enzyme Mix (50X)	40 µl
Nuclease-free H2O	1 ml

Product Description

Green-2-Go 1-Step RT-qPCR Mastermix-No Dye Kit is a complete qPCR system containing all necessary reagents for both reverse transcription and PCR amplification to occur in a single qPCR reaction tube. Specifically, this Green-2-Go 1-Step RT-qPCR Mastermix-No Dyer kit contains a qRT-PCR Enzyme Mix and a Green-2-Go 1-Step RT-qPCR Mastermix-No Dye. Our proprietary qRT-PCR Enzyme Mix contains stabilizers and enhancers to optimize the two reactions in a real-time “single step”. This Green-2-Go 1-Step RT-qPCR Mastermix-No Dye kit offers the end-users an efficient, easy to use and reliable alternative to conventional “two-step” sequential qRT-PCR. Gene-specific primers must be used along with this kit.

Storage Conditions

Store all components at -20°C in a non-frost-free freezer. All components are stable for 1 year from the date of shipping when stored and handled properly.

Protocol

RT-PCR should be assembled in a nuclease-free environment. RNA sample preparation, reaction mixture assembly, PCR and subsequent reaction analysis should be performed in separate areas. The use of “clean”, automatic pipettors designated for PCR and aerosol resistant barrier tips are recommended.

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

Components	Reaction Volume			Concentration
	10 µl	20 µl	50 µl	
Total RNA or Poly(A) + mRNA	Variable	Variable	Variable	2 pg - 0.2 µg/rxn 0.01 pg - 2 ng/rxn
Green-2-Go qPCR Mastermix-No Dye	5 µl	10 µl	25 µl	1X
qRT-PCR Enzyme Mix (50X)	0.2 µl	0.4 µl	1 µl	1X
Forward Primer (6 µM)	0.5 µl	1 µl	2.5 µl	300 nM
Reverse Primer (6 µM)	0.5 µl	1 µl	2.5 µl	300 nM
Nuclease-free H2O	Up to 10 µl	Up to 20 µl	Up to 50 µl	-

Note: *1. Gene specific primers must be used.*
2. Amplicon should be <150 bp in size.

1. Gently mix and ensure all the components are at the bottom of the amplification tube. Centrifuge briefly if needed.
2. Program the thermal cycler so that cDNA synthesis is followed immediately by qPCR amplification.

Step	Temperature	Duration	Cycle(s)
cDNA Synthesis	42°C	15 min	1
Pre-Denaturation	95°C	10 min	1
Denaturation	95°C	15 sec	40
Annealing	60°C	60 sec	
Melt Curve	According to the instrument guidelines		

Recommendations for Optimal Results

Aliquot reagents to avoid contamination and repeated freeze-thaw cycles.
Green-2-Go qPCR Mastermix- No Dye components are light sensitive; avoid prolonged exposure to intense light.
Start reaction as soon as the reaction mixture is prepared and always keep the reaction mixture chilled in an ice box prior to qRT-PCR reaction.

For laboratory research only. Not for clinical applications.

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