

## Product information

 QF 24 TV4  
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# Green-2-Go 1-Step qRT-PCR Mastermix Kit

**Catalog #:** QPCR012-LR | QPCR011-iC  
**Size:** 100 Rxn (1.25ml)  
**Storage:** -20°C

### Description:

Bio Basic's 1-Step Green-2-Go qRT-PCR 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 1-Step qRT-PCR kit contains a qRT-PCR Enzyme Mix and a Green-2-Go qPCR MasterMix. Our proprietary qRT-PCR Enzyme Mix contains stabilizers and enhancers to optimize the two reactions in a real-time "single step". This 1-Step qRT-PCR kit offers 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.

### Components:

Part No.	Components	Cat. No.	
		QPCR012-LR	QPCR011-iC
QPCR012-LR	Green-2-Go 1-step qPCR MasterMix - Low ROX	1.25 ml	-
QPCR011-iC	Green-2-Go 1-step qPCR MasterMix - iCycler	-	1.25 ml
qRT-PCR-050	qRT-PCR Enzyme Mix (50X)	40 µl	
Nuclease Free H <sub>2</sub> O	Nuclease-free H <sub>2</sub> O	1 ml	
<b>Size</b>		<b>100x20 µl reactions</b>	

### 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.

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	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 H <sub>2</sub> O	Up to 10 µl	Up to 20 µl	Up to 50 µl	-

#### Note:

- Gene specific primers must be used.
- Amplicon should be <150 bp in size.
- Please ensure no salt crystals are present in the Green-2-Go 2X qPCR MasterMix before use. If salt crystals are observed, mix until crystals are completely dissolved and absent.

2. Gently mix and ensure all the components are at the bottom of the amplification tube.  
Centrifuge briefly if needed.

3. Program the thermal cycler so that cDNA synthesis is followed immediately by qPCR amplification.

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

### Recommendations for Optimal Results:

- Aliquot reagents to avoid contamination and repeated freeze-thaw cycles.
- Green-to-go qPCR MasterMix 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.

### 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.



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