HiSense™ QGreenBlue RT-qPCR Master Mix

Cat. No. RTQBLR-01, RTQBLR-05, RTQBHR-01, RTQBHR-05

1. Product Information

Introduction

The HiSense™ QGreenBlue RT-qPCR Master Mix is specifically designed for the reverse transcription and PCR steps, which are performed in a one-step assay in a single tube. This specialized mix comprises MMLV reverse transcriptase, Taq Polymerase, and essential components (excluding primers, RNA template, and water) required for RT-qPCR.

Product Description

The HiSense™ QGreenBlue RT-qPCR Master Mix contains enough reagents for 500 experiments based on a 20 µl reaction at 2x concentration. Adding RNA templates and primers directly to a single tube will facilitate the two processes, saving time and minimizing the risk of contamination. The qPCR results are represented by measuring the real-time fluorescence intensity resulting from the binding of SybrGreen Type dye to double-stranded (ds) DNA. This master mix combines M-MLV RTase with Taq DNA polymerase to ensure stable performance and accurate quantification of gene expression.

2. Contents and Storage

Materials Provided

Label	RTQBLR-01	RTQBLR-05
2X QGreenBlue RT-qPCR Master Mix (Low ROX*)	1 ml	5 ml
Label	**RTQBHR-01**	RTQBHR-05
2X QGreenBlue RT-qPCR Master Mix (High ROX*)	1 ml	5 ml

* Cat. No for Instruments (Depending on ROX concentration)
: See [Appendix A]

Storage

Store at -20°C

Check the label on the product for expiration date.

3. Test Protocol

Preparing for qPCR reaction

Before use, mix the 2X RT-QGreenBlue qPCR Master Mix well by inverting the tube several times and centrifuge it.

Do not vortex!! - Inverting and tapping are enough to mix.

1. Prepare a reaction master mix by adding the following components (except template RNA) for each 20µl reaction to a tube at room temperature.

Reaction components	Volume
2X QGreenBlue RT-qPCR Master Mix	10 µl
Forward primers, (10pmol/µl)*	1-2 µl
Reverse primers, (10pmol/µl)*	1-2 µl
Template RNA	≤ 500 ng
Nuclease free water	up to 20µl
Total volume	20µl

* A final primer concentration of 0.2 µM is optimal in most cases but may be individually optimized in a range of 0.1 µM to 1.0 µM.

2. Gently mix (do not vortex) the master mix by inverting, centrifuge briefly and dispense appropriate volumes into PCR tubes or plates.

3. Add template RNA to the individual PCR tubes or wells containing the master mix.

4. Centrifuge briefly.

5. Program the thermal cycler according to the recommendations below, place the samples in the cycler and start the program.

Thermal Cycling Condition

1. Two-step cycling protocol (Fast)

Steps	Temp(℃)	Time	Cycles
Reverse Transcription	42	10 min	1
Pre-denature	95	1 min	1
Denature	95	5 sec	40
Anneal / Extend and read fluorescence	60	10~30 sec*	
Melting curve analysis depending on Instrument manual			

* An optimal time may be optimized depending on amplicon size.

2. Three-step cycling protocol

Steps	Temp(℃)	Time	Cycles
Reverse Transcription	42	10 min	1
Pre-denature	95	1 min	1
Denature	95	5 sec	40
Anneal	55~65	10~30 sec*	
Extend and read fluorescence	72	10~30 sec*	
Melting curve analysis depending on Instrument manual			

* An optimal time may be optimized depending on amplicon size.

[Appendix A]

Cat. No for Instruments (Depending on ROX concentration)

Instruments		Cat. No.
Brand	Model	
BioRad	iCycler, MyiQ, MiQ 2, iQ 5, CFX-96, CFX-384	(No ROX) RTQBLR
MJ Research	Opticon, Option2, Chromo4, MiniOpticon	
Qiagen	Roto-Gene Q, Roto-Gene3000, Roto-Gene 6000	
Eppendorf	Mastercycler realplex	
Illumina	Eco RealTime PCR System	
Roche	LightCycler 480, LightCycler 2.0	
ABI	5700, 7000, 7300, 7700, 7900, 7900HT, 7900HT Fast, StepOne, StepOne plus	(High ROX) RTQBHR
ABI	7500, 7500 Fast, QuantStudio (1, 3, 5, 6, 7)	(Low ROX) RTQBLR
Stratagene	MX3000, MX3005P, MX4000	