

# HiSense™ QPlex RT-qPCR Master Mix

Cat. No. RTQP-01, RTQP-05

## 1. Product Information

### Introduction

HiSense™ QPlex RT-qPCR Master Mix is a one-tube, one-step, ready-to-use master mix for RNA quantification with hydrolysis probe detection. The optimized buffer formulation offers specificity, robustness, and reliability. One-Step RT-qPCR uses gene specific primers, designed to match RNA/cDNA targets, in a single-tube/plate, one-step reaction. This approach offers tremendous convenience when applied to analysis of single targets from multiple RNA samples. Also, it minimizes the possibility of introducing contaminants into reactions between the RT and PCR steps, since both steps are carried out sequentially without opening the reaction tubes/plates between the steps designed specifically for real-time PCR with hydrolysis probe.

### Product Description

HiSense™ QPlex qPCR Master Mix is supplied in a 2X concentration. The mix is optimized for hydrolysis probe RT-qPCR and contains HotTaq DNA Polymerase, RTase, dNTPs, and optimized buffer components.

## 2. Contents and Storage

### Materials Provided

Label	RTQP-01	RTQP-05
2X QPlex RT-qPCR Master Mix	1 ml	5 ml
50X ROX (reference dye)	40 µl	200 µl

### 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 QPlex RT-qPCR Master Mix well by inverting the tube several times.

**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 QPlex RT-qPCR Master Mix	10 µl
Forward primers, (10pmol/µl)*	0.5 µl
Reverse primers, (10pmol/µl)*	0.5 µl
Probe**	1 µl
50X ROX*	0X, 0.1X and 1X
Template RNA***	2 µl
Nuclease free water	up to 20µl
Total volume**	20µl

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

\*\* Probe concentration differs depending on the real-time PCR instrument and type of fluorescent label. Refer to the instrument operation manual and product documents supplied with the probe.

\*\*\* Use total RNA as template range of 10 pg-100 ng.

**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

Steps	Temp(°C)	Time	Cycles
Reverse Transcription*	42	10 min	1
Pre-denature	95	5 min	1
Denature	95	20 sec	40
Anneal / Extend**	60	60 sec	

\* The reverse transcription time can be increased by 10 to 60minutes or more, depending on the size of the template RNA.

\*\* Data acquisition should be performed during the anneal / extend step. Optimal Anneal / Extend temperature depends on the melting temperature of the primers.

### 2. Three-step cycling protocol

Steps	Temp(°C)	Time	Cycles
Reverse Transcription*	42	10 min	1
Pre-denature	95	5 min	1
Denature	95	20 sec	40
Anneal**	60	30 sec	
Extend***	72	30 sec	

\* The reverse transcription time can be increased by 10 to 60minutes or more, depending on the size of the template RNA.

\*\*Optimal Annealing temperature depends on the melting temperature of the primers.

\*\*\* Data acquisition should be performed during the extend step.

## [Appendix A]

### ROX concentration for Instruments

Instruments		Cat. No.
Brand	Model	
BioRad	iCycler, MyiQ, MiQ 2, iQ 5, CFX-96, CFX-384	No ROX (0X)
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 (1X)
ABI	7500, 7500 Fast, QuantStudio (3, 5, 7)	Low ROX (0.1X)
Stratagene	MX3000, MX3005P, MX4000	