HiSense™ QPlex qPCR Master Mix

Cat. No. QP-05

1. Product Information

Introduction

HiSense™ QPlex qPCR Master Mix is designed specifically for real-time PCR with probe. This mix contains HotTaq DNA Polymerase and components (except primers, template and water) necessary to perform real-time PCR using probe. Direct detection of PCR product is monitored by measuring the increase in fluorescence releasing from probe.

Product Description

HiSense™ QPlex qPCR Master Mix is supplied in a 2X concentration and contains sufficient reagents to perform reactions. The mix is optimized for Probe reactions and contains HotTaq DNA Polymerase, dNTPs, passive reference, and optimized buffer components.

2. Contents and Storage

Materials Provided

Label	QP-05
2X QPlex qPCR Master Mix	5 ml
50X ROX (reference dye)	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 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 DNA) for each 20µl reaction to a tube at room temperature.

Reaction components	Volume
2X QPlex 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 DNA***	2 µl
DNase free water	up to 20µl
Total volume	20µl

*A final primer concentration of 0.2 µM is most likely to yield good results. However, should further optimization be required, try adjusting primer concentrations in the range of 0.1 to 1.0 µM.

**The probe concentration varies depending on the real-time PCR instrument being used and the type of fluorescent label.

Refer to the instrument manual and the probe data sheet to determine the appropriate concentration.

*** The optimal quantity varies depending on the number of target copies present in the template solution. Use no more than 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 DNA 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(℃)	Time	Cycles
Pre-denature	95	5 min	1
Denature	95	20 sec	40
Anneal / Extend*	60	60 sec	

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

2. Three-step cycling protocol

Steps	Temp(℃)	Time	Cycles
Pre-denature	95	5 min	1
Denature	95	20 sec	40
Anneal*	60	30 sec	
Extend**	72	30 sec	

*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	