

HiSense™ RT-PCR Premix

Cat. No. LRTP-96, LRTP-480

1. Product Information

Introduction

HiSense™ RT-PCR Premix is designed to perform a PCR reaction easily by dispensing HiSense™ RT-PCR Master Mix into an 8-strip tube. It was dried by vacuum freezing drying method so that the user can use it immediately by adding a template, primers, and water.

This product offers a single-step procedure for performing RT-PCR reactions. Subsequent loading and visualization of RT-PCR products are streamlined since the loading dye are included. The user supplies the primers and DNase-treated template RNA.

The Premix contains all necessary reagents for PCR amplification to occur in a single reaction tube. Specifically, this One-Step RT-PCR master mix contains special reaction buffer, RTase Inhibitor, dNTPs and DNA-free Taq DNA Polymerase for highly sensitive and specific RT-PCR using any RNA template. The special RT-PCR buffer contains stabilizers and enhancers that optimize the two reactions in a “single step”.

2. Contents and Storage

Materials Provided

Label	LRTP-96	LRTP-480
2X RT-PCR Premix	8-Strip × 12ea	8-Strip × 60ea

Storage

Store at -20°C

Check the label on the product for expiration date.

3. Test Protocol

Reaction mixture (for 20µl reaction)

Reaction components	Volume
2X RT-PCR Premix	-
Forward primers, (10pmol/µl)*	1 µl
Reverse primers, (10pmol/µl)*	1 µl
Template RNA**	2 µl
Nuclease free water	up to 20 µl
Total volume**	20 µl

* 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.

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

After tapping the 8-strip tube containing the above reaction mixture, briefly spin-down it. (Repeat until the reaction mixture is completely dissolved.)

PCR reaction condition

Steps & Cycles	Temp(°C)	Time	Cycles
Reverse Transcript*	42	15 min	1
Pre heat	95	5 min	1
Denature	95	30 sec	30~40
Anneal**	60	30 sec	
Extend***	72	1 min	
Final extension	72	5 min	1

* The reverse transcription time can be increased by 30 to 60 minutes or more, depending on the size of the template RNA.

**Optimal annealing temperature depends on the melting temperature of the primers.

*** Generally, 1 min/kb and higher than 3 kb, set it to 1.5 to 2.0 min/kb.