



# Hifair™ Lyo Multiplex One Step RT-qPCR Kit

## Product description

Hifair™ Lyo Multiplex One Step RT-qPCR Kit is a multiplex quantitative PCR kit based on RNA as template. In the process of the experiment, reverse transcription and quantitative PCR were carried out in the same tube, which simplified the experimental operation and reduced the risk of contamination. The unique Buffer and enzyme design ensures that it can be used in the preparation and product design of one-step RT-qPCR lyophilized reaction systems.

In this kit, the first strand cDNA was efficiently synthesized by heat-resistant Hifair™ V Reverse Transcriptase and quantitatively amplified by Unicon™ HotStart Taq DNA Polymerase. The kit mainly contains optimized lyophilizable buffer, enzymes mix, etc. In addition, the factors that can effectively inhibit the non-specific PCR amplification and improve the amplification efficiency of multiple qPCR reactions are added, which can ensure the amplification efficiency and carry out up to multiple amplification reaction.

This kit is fitted with lyoprotectant that can be used for lyophilization.

## Components

Components No.	Name	11831ES60 (100T)	11831ES80 (1,000T)	11831ES92 (10,000T)
11831-A	4× Hifair™ V Lyo-Buffer	600 µL	6 mL	60 mL
11831-B	Hifair™ Lyo-Enzyme Mix	100 µL	1 mL	10 mL
11831-C	Lyoprotectant	800 µL	8 mL	80 mL

## Specifications

Hot Start	Built-in hot start
Detection method	Primer-probe detection
PCR method	One step RT-qPCR
Type of sample	RNA
Application equipment	<p><b>Equipment with Rox:</b> ABI 5700, 7000, 7300, 7700, 7900HT Fast, StepOne™, StepOne Plus™</p> <p><b>Equipment with Low Rox:</b> ABI 7500, 7500 Fast, ViiA™7, QuantStudio™ 3 and 5, QuantStudio™ 6,7,12k Flex</p> <p><b>Stratagene</b> MX3000P™, MX3005P™, MX4000P™</p> <p><b>Equipment without Rox:</b></p> <p><b>Bio-Rad</b> CFX96™, CFX384™, iCycler iQ™, iQ™ 5, MyiQ™, MiniOpticon™, Opticon®, Opticon® 2, Chromo4™</p> <p><b>Eppendorf</b> Mastercycler® ep realplex, realplex 2 s; <b>Qiagen</b> Corbett Rotor-Gene® Q, Rotor-Gene® 3000, Rotor-Gene® 6000</p> <p><b>Roche Applied Science</b> LightCycler® 480, LightCycler® 2.0, Lightcycler® 96</p>



## Storage

Part 1: 11831-A [4×Hifair V Lyo-Buffer] should be stored at -25°C ~ -15°C for 1 year.

Part 2: 11831-B [Hifair™ Lyo-Enzyme Mix] should be stored at 2°C ~ 8°C for 6 months.

11831-C [Lyoprotectant] should be stored at -25°C ~ -15°C for 1 year.

## Instructions

### 1. Reaction Composition

Components	Volume (μL)	Final Concentration
4×Hifair™ V Lyo-Buffer	6	1×
Hifair™ Lyo-Enzyme Mix	1	-
Lyoprotectant	8	-
Primer Mix (10 μM)	1	0.1-1 μM
Probe Mix (10 μM)	0.5	0.05-0.5 μM
Template RNA	5	-
DEPC H <sub>2</sub> O	to 25	-

Note: Be sure to mix well before use, avoid excessive bubbles caused by violent vibration.

### 2. Optimized Cycling Protocol

	Reaction stage	Temperature	Time	Cycle
1	Reverse transcription	50°C <sup>a</sup>	10 min	1
2	Initial denaturation	95°C	2 sec	1
3	Amplification reaction	95°C	1 sec	45 cycles
		60°C <sup>b</sup>	13 sec <sup>c</sup>	

Note:

a) Reverse transcription: The temperature can select 42°C or 50°C for 10-15 minutes.

b) Amplification reaction: The temperature is adjusted according to the T<sub>m</sub> value of the designed primers.

c) Fluorescence signal acquisition: Please set the experimental procedure according to the requirements of the instrument manual.