Hifair™ V Multiplex One Step RT-qPCR Probe Kit（UDG Plus）

Product description

Hifair™ V Multiplex One Step RT-qPCR Probe Kit (UDG Plus) 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.

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 MP buffer, enzymes mix, etc. The buffer solution already contains Mg^{2+} and dNTP. 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. The dUTP/UDG system was added to effectively prevent the risk of aerosol contamination.

Components

Components No.	Name	13650ES50 (50 T)	13650ES60 (100 T)	13650ES80 (1,000 T)	13650ES92 (10,000 T)
13650-A	2×Hifair™ V MP Buffer	750 μL	1.5 mL	15 mL	150 mL
13650-B	Hifair™ V Enzyme Mix	60 μL	120 μL	1.2 mL	12 mL

Note: 1) 2×Hifair™ V MP Buffer is the abbreviation of Hifair™ V Multiplex One Step RT-qPCR Probe Buffer, which includes dNTPs, dUTP, Mg^{2+}, stabilizers, enhancers, and more.

2) Hifair™ V Enzyme Mix mainly contains heat-resistant Hifair™ V reverse transcriptase, UNICON™ HotStart Taq DNA polymeras and UDGase.

Specifications

PCR method	One step RT-qPCR
Type of sample	RNA
Application equipment	**Equipment with Rox**： **ABI** 5700, 7000, 7300, 7700, 7900HT Fast, StepOne™, StepOne Plus™ **Equipment with Low Rox**： **ABI** 7500, 7500 Fast, ViiA™7, QuantStudio™ 3 and 5, QuantStudio™ 6,7,12k Flex **Stratagene** MX3000P™, MX3005P™, MX4000P™ **Equipment without Rox**: **Bio-Rad** CFX96™, CFX384™, iCycler iQ™, iQ™5, MyiQ™, MiniOpticon™, Opticon®, Opticon® 2, Chromo4™ **Eppendorf** Mastercycler® ep realplex, realplex 2 s; **Qiagen** Corbett Rotor-Gene® Q, Rotor-Gene® 3000, Rotor-Gene® 6000 **Roche Applied Science** LightCycler® 480, LightCycler® 2.0, Lightcycler® 96 **Thermo Scientific** PikoReal Cycler; **Cepheid** SmartCycler®; **Illumina** Eco qPCR

Storage

The product should be stored at -25°C~-15°C for 1 year.

Instructions

1. Reaction Composition

Components	Volume (μL)	Final Concentration
2×Hifair™ V MP Buffer	15	1×
Hifair™ V Enzyme Mix	1.2	-
Primer / Probe Mix (2.5 μM)	3	0.25 μM
Template RNA	1-10	-
RNase Free H_2O	to 30	-

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

a) Primer concentration: Primer mix including multiplex primer, depending on the situation optimal primer concentration may be between 0.1 and 1.0 μM.

b) Probe concentration: Probe mix including multiplex probe labeling difference fluorescent group, depending on the situation optimal probe concentration may be between 0.05 and 0.5 μM.

c) Template dilution: qPCR is highly sensitive and it is recommended to dilute the template. The control Ct value is suitable between 20 and 35.

d) System preparation: un head with filter element. Avoid cross contamination and aerosol contamination.

2. Optimized Cycling Protocol

	Reaction stage	Temperature	Time	Cycle
1	Reverse transcription	50°C[a]	10 min	1
2	Initial denaturation	95°C	5 min	1
3	Amplification reaction	95°C	15 sec	45 cycles
		60°C[b]	30 sec[c]	

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 Tm value of the designed primers.

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