



Ver. HB221115

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 Mg²⁺ 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	13747ES50 (50T)	13747ES60 (100T)	13747ES80 (1,000T)	13747ES92 (10,000T)
13747-A	2×Hifair™ V MP Buffer	625 μL	1.25 mL	12.5 mL	125 mL
13747-B	Hifair™ V Enzyme Mix	50 μL	100 μL	1 mL	10 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>Equipment with Rox: ABI 5700, 7000, 7300, 7700, 7900HT Fast, StepOne™, StepOne Plus™</p> <p>Equipment with Low Rox: ABI 7500, 7500 Fast, ViiA™7, QuantStudio™ 3 and 5, QuantStudio™ 6,7,12k Flex</p> <p>Stratagene MX3000P™, MX3005P™, MX4000P™</p> <p>Equipment without Rox:</p> <p>Bio-Rad CFX96™, CFX384™, iCycler iQ™, iQ™5, MyiQ™, MiniOpticon™, Opticon®, Opticon® 2, Chromo4™</p> <p>Eppendorf Mastercycler® ep realplex, realplex 2 s; Qiagen Corbett Rotor-Gene® Q, Rotor-Gene® 3000, Rotor-Gene® 6000</p> <p>Roche Applied Science LightCycler® 480, LightCycler® 2.0, Lightcycler®96</p> <p>Thermo Scientific PikoReal Cyclyer; Cepheid SmartCycler®; Illumina Eco qPCR</p>



Shipping and Storage

The product is shipped with dry ice and can be stored at -15°C ~ -25°C for 1 year. It should avoid repeated freezing and thawing. It is recommended to save separately.

Instructions

1. Reaction Composition

Components	Volume (μL)	Final Concentration
2× Hifair™ V MP Buffer	12.5	1×
Hifair™ V Enzyme Mix	1	-
Primer/Probe Mix (2.5 μM)	3	0.3 μM
Template RNA	1-10	-
RNase Free H ₂ O	to 25	-

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: Please prepare in the ultra-clean working table, and pipettor and reaction tube without nuclease residue; it is recommended to use the gun 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	20 min	1
2	Initial denaturation	95°C	5 min	1
3	Amplification reaction	95°C	15 sec	40-45 cycles
		60°C ^b	30 sec ^c	

Note:

a) Reverse transcription: The temperature can select 42°C or 50°C.

b) Amplification reaction: The temperature is adjusted according to the T_m value of the designed primers.

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

Notes

Please wear the necessary PPE, such lab coat and gloves, to ensure your health and safety!