HB220429

Hieff Unicon™ nCov Multiplex One Step RT-qPCR Probe Kit

Product Information

Product name	Cat#	Size
Hieff Unicon™ nCov Multiplex One Step RT-qPCR Probe Kit	13173ES60	100 T
	13173ES80	1000 T
	13173ES92	10000 T

Product Description

The Hieff Unicon™ nCov Multiplex One Step RT-qPCR Probe Kit is a kit for performing multiplex quantitative PCR reactions using RNA as template. During the experiment, reverse transcription and quantitative PCR are performed in the same reaction tube, simplifying the experiment and reducing the risk of contamination.

The kit uses heat resistant Hifair™ V Reverse Transcriptase for efficient synthesis of first strand cDNA and UNICON™ HotStart Taq DNA Polymerase for quantitative amplification. The kit mainly contains optimized MP Buffer, Enzymes Mix, etc. The MP buffer already contains Mg^{2+} and dNTPs, etc. Factors that effectively inhibit non-specific amplification and factors that enhance the amplification efficiency of multiplex qPCR reactions have been added, enabling up to four reactions to be performed while ensuring primer amplification efficiency.

Product Component

Component Number	Components	Cat#/Size		
		13173ES60 (100 T)	13173ES80 (1000 T)	13173ES92 (10000 T)
13173-A	2× Hifair™ V MP Buffer	1.25 mL	12.5 mL	125 mL
13173-B	Hifair™ V Enzyme Mix	100 µL	1 mL	10 mL

[Notes]:

1. 2× Hifair™ V MP Buffer is an abbreviation for Multiplex One Step RT-qPCR Probe Buffer.
2. Hifair™ V Enzyme Mix contains mainly Hifair™ V Reverse Transcriptase and UNICON™ HotStart Taq DNA Polymerase.

Applicable Models

Equipment with Rox: **ABI** 5700, 7000, 7300, 7700, 7900HT Fast, StepOne, StepOne Plus

Equipment with low Rox: **ABI** 7500, 7500 Fast, ViiA7, QuantStudio 3 and 5, QuantStudio 6,7,12k Flex

Stratagene MX3000P, MX3005P, MX4000P

Equipment without Rox:

Bio-Rad CFX96, CFX384, iCycler iQ, iQ5, 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

Shipping and Storage

The product is shipped with ice packs and can be stored at -20°C for 2 years. Please avoid repeated freeze-thaw. Recommend for storage in separate packs.

Cautions

1. Please use RNase free consumables during the experiment.
2. For your safety and health, please wear lab coats and disposable gloves for operation.

Reaction System (25 μL Volume)

Components	Volume (μL)	Final Concentration
2× Hifair™ V MP Buffer	12.5	1×
Hifair™ V Enzyme Mix	1	-
Primer Mix (10 μmol/L)	1 each	0.25 μmol/L
Probe Mix (10 μmol/L)	0.25 each	0.1 μmol/L
Template RNA	1-10	-
RNase Free H_2O	to 25	-

[Notes]: Be sure to mix well before use to avoid excessive air bubbles from violent shaking.

1. **Primer concentration:** Primer Mix contains several pairs of primers and the final concentration of each primer can usually be adjusted between 0.1 and 1.0 μmol/L depending on the situation.
2. **Probe concentration**: The Probe Mix contains several probes with different fluorescent signals, each with a concentration adjustable between 50-300 nmol/L depending on the situation.
3. **Template dilution:** qPCR is extremely sensitive and it is recommended that the template is diluted and used with a controlled Ct of between 20-35.
4. **Reaction system:** 25-50 μL is recommended to ensure the validity and reproducibility of the target gene amplification.
5. **System preparation:** Please prepare the system in an ultra-clean bench and use pipette tips and reaction tubes without nuclease residues; pipette tips with filter are recommended. Avoid cross-contamination and aerosol contamination.

Standard Amplification Protocol

Cycle Step	Temperature	Time	Cycles
RT incubation	50°C[a]	10 min	1
Enzyme activation	95°C	5 min	1
Amplification	95°C	15 sec	45
	58°C[b]	30 sec[c]	

[Notes]:

a) Reverse transcription: either 42°C or 50°C.

b) Amplification: The amplification reaction temperature is adjusted according to the designed primer Tm values.

c)Fluorescence signal acquisition: Different qPCR detection instruments require different fluorescence signal acquisition times, please set according to the minimum time limit.