

## Hifair™ V Multiplex One Step RT-qPCR Probe Kit

### Product Information

| Product Name                                   | Cat#      | Size    |
|------------------------------------------------|-----------|---------|
| Hifair™ V Multiplex One Step RT-qPCR Probe Kit | 11147ES50 | 50 T    |
|                                                | 11147ES70 | 200 T   |
|                                                | 11147ES80 | 1000 T  |
|                                                | 11147ES92 | 10000 T |

### Product Description

Hifair™ V Multiplex One-Step RT-qPCR Probe Kit is for multiple quantitative PCR reactions with RNA as a template. During the experiment, the reverse transcription and quantitative PCR are carried out in the same reaction tube, simplified experimental operation and reduced the risk of pollution.

The kit uses heat-resistant Hifair™ V Reverse Transcriptase to efficiently synthesize the first chain cDNA, and use Unicon™ Hotstart Taq DNA Polymerase to quantify quantitative amplification. The kit mainly includes an optimized MP Buffer, Enzymes Mix, etc., which already contains Mg<sup>2+</sup> and DNTP, etc., and has added factors that effectively inhibit non-specific PCR amplification factors and enhance multiple qPCR reactive amplification efficiency, which can be guaranteed at the same time of primer amplification, up to four reactions.

### Product Components

| Component Name | Components                  | Cat#/Size           |                      |                       |                        |
|----------------|-----------------------------|---------------------|----------------------|-----------------------|------------------------|
|                |                             | 11147ES50<br>(50 T) | 11147ES70<br>(200 T) | 11147ES80<br>(1000 T) | 11147ES92<br>(10000 T) |
| 11147-A        | 2×Hifair™ V MP Buffer       | 500 µL              | 2 × 1 mL             | 10 mL                 | 100 mL                 |
| 11147-B        | Hifair™ V Enzyme Mix        | 50 µL               | 200 µL               | 1 mL                  | 10 mL                  |
| 11147-C        | RNase Free H <sub>2</sub> O | 500 µL              | 2 × 1 mL             | 10 mL                 | 100 mL                 |

[Notes]: 1) 2 × Hifair™ V MP Buffer is short of Multiplex One Step RT-qPCR Probe Buffer.

2) Hifair™ V Enzyme Mix mainly includes heat-resistant Hifair™ V Reverse Transcriptase and Unicon™ Hotstart Taq DNA Polymerase.

### Applicable Models

**ABI Series:** ABI 5700, 7000, 7300, 7700, 7900HT Fast, StepOne, StepOne Plus; 7500, 7500 Fast, ViiA7, QuantStudio 3 and 5, QuantStudio 6, 7, 12k Flex;

**Bio-Rad Series:** Bio-Rad CFX96, CFX384, iCycler iQ, iQ5, MyiQ, MiniOpticon, Opticon, Opticon 2, Chromo4;

**Roche Series:** Roche Applied Science LightCycler 480, LightCycler 2.0; Lightcycler 96;

**Others:** Stratagene MX3000P, MX3005P, MX4000P;

**Eppendorf** Mastercycler ep realplex, realplex 2 s;

**Qiagen** Corbett Rotor-Gene Q, Rotor-Gene 3000, Rotor-Gene 6000;

**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 one year. Please avoid repeated freeze-thaw.

### Cautions

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

### Reaction System (Take 20 $\mu$ L for example)

| Components                                  | Volume           | Final Concentration |
|---------------------------------------------|------------------|---------------------|
| 2 $\times$ Hifair <sup>TM</sup> V MP Buffer | 10 $\mu$ L       | 1 $\times$          |
| Hifair <sup>TM</sup> V Enzyme Mix           | 1 $\mu$ L        | -                   |
| Primer Mix (10 $\mu$ mol/L)                 | 0.4 $\mu$ L each | 0.2 $\mu$ mol/L     |
| Probe Mix (10 $\mu$ mol/L)                  | 0.2 $\mu$ L each | 0.1 $\mu$ mol/L     |
| Sample RNA                                  | 1 pg-1 $\mu$ g   | -                   |
| RNase Free H <sub>2</sub> O                 | To 20 $\mu$ L    | -                   |

[Notes]: Be sure to mix well before use to avoid excessive air bubbles caused by vigorous shaking.

- 1) Primer Concentration:** Primer Mix contains multiple pairs of primers, usually the final concentration of each primer is 0.2  $\mu$ mol/L, and can also be adjusted between 0.1-1.0  $\mu$ mol/L according to the situation;
- 2) Probe Concentration:** Probe Mix contains multiple probes with different fluorescent signals, and the concentration of each probe can be adjusted between 50-300 nmol/L according to the specific situation;
- 3) Template Dilution:** The sensitivity of qPCR is extremely high. It is recommended to dilute the template and control the Ct value between 20-35;
- 4) Reaction System:** 20  $\mu$ L or 50  $\mu$ L is recommended to ensure the validity and repeatability of target gene amplification;
- 5) System Preparation:** Please configure in the ultra-clean workbench, and use pipette tips and reaction tubes without nuclease residues; it is recommended to use pipette tips with filter elements. Avoid cross-contamination and aerosol contamination;

### Standard Amplification Procedure

| Cycle Step             | Temperature       | Time                | Cycles |
|------------------------|-------------------|---------------------|--------|
| Reverse Transcription  | 50°C <sup>a</sup> | 15 min              | 1      |
| Initial denaturation   | 95°C              | 30 sec              | 1      |
| Amplification Reaction | 95°C              | 10 sec              | 45     |
|                        | 60°C <sup>b</sup> | 30 sec <sup>c</sup> |        |

[Notes]:

- a) Reverse Transcription:** For some complex templates or RNA templates with high GC content, the reverse transcription temperature can be adjusted to 55°C, which is beneficial to improve the amplification efficiency;
- b) Amplification Reaction:** The temperature of the amplification reaction is adjusted according to the T<sub>m</sub> value of the designed primers;
- c) Fluorescence Signal Collection:** Different qPCR detection instruments require different fluorescence signal collection times, please set according to the shortest time limit.