



## HiScript Multiplex Probe One Step qRT-PCR Kit(UDG)

Product Number: PCK005

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### Shipping and Storage

-20°C.

### Components

| Component                           | PCK005 |
|-------------------------------------|--------|
| 5×HiScript Multiplex qRT-PCR buffer | 500µl  |
| HiScript Multiplex RT Enzyme Mix    | 125µl  |
| RNase Free Water                    | 1ml    |
| ROX I                               | 50µl   |
| ROX II                              | 50µl   |

1. The Real Time PCR instrument calibrated with ROX I includes:  
ABI 5700/7000/7300/7700/7900/7900 HT/7900 HT Fast,ABI StepOne/StepOnePlus and other instruments.
2. The Real Time PCR instrument calibrated with ROX II includes:  
ABI 7500/7500 Fast;ABI ViiA7;ABI Q6,ABI Quant Studio 6/7 Flex;Stratagene MX4000/MX3005P/MX3000P and other instruments.
3. Real Time PCR instruments that do not require ROX calibration include:  
Bio-Rad CFX96/CFX 384/iCycler iQ5/iQ/My iQ5/MiniOpticon/Opticon/Opticon 2/Chromo4 ; Eppendorf Mastercycler ep realplex /realplex 2s; Cepheid SmartCycler; Illumina Eco qPCR; Roche Applied Science LightCycler 480; Thermo Scientific PikoReal Cycler; Qiagen/Corbett Rotor-Gene Q/Rotor-Gene 3000/ Rotor-Gene 6000 and other instruments.

### Description

HiScript Multiplex Probe One Step qRT PCR Kit (UDG) is a one-step quantitative detection kit using RNA as a template. This kit can use total RNA or mRNA from animals, plants, and microorganisms as templates, reverse transcribe them into cDNA, perform qPCR amplification using gene specific primers, and quantify the fluorescence value of the detection probe. CDNA synthesis and qPCR reaction are carried out in one tube, and after reverse transcription, qPCR amplification can be directly performed without adding additional steps, which helps with the processing of large amounts of samples and reduces the chance of contamination. And multiple pairs of primers can be added to perform multiple amplification on multiple genes in one tube. This reagent kit is composed of Taq DNA polymerase, Mg<sup>2+</sup>, dNTPs, etc. that have undergone special processing, combined with carefully optimized reaction buffer, so that all primers in the reaction system can be effectively extended without additional optimization, and accurate quantification can be carried out over a wider range. Adding a dUTP/UDG pollution prevention system to the system can effectively degrade U containing pollutants and reduce false positives.

### Features

1. High sensitivity: The sensitivity of the detection standard can reach 10 copies;
2. Anti pollution system: Adding dUTP/UDG anti pollution system to the system can effectively degrade U containing pollutants and reduce false positives;
3. Multiple detection: 4-6 targets can be detected simultaneously in the same reaction tube.

### Protocol

1. Common reaction systems (25µl):

|                                     |        |
|-------------------------------------|--------|
| 5×HiScript Multiplex qRT-PCR buffer | 5µl    |
| HiScript Multiplex RT Enzyme Mix    | 1.25µl |



# Tinzyme Co., Limited

Email: [sales@tinzyme.com](mailto:sales@tinzyme.com)

Website: [www.tinzyme.com](http://www.tinzyme.com)

Tel: +86-755-86134126

WhatsApp/Facebook/Twitter: +86-189-22896756

|   |                              |
|---|------------------------------|
| Upstream primer                             | 0.2-1.0 $\mu$ M(Final Conc.) |
| Downstream primer                           | 0.2-1.0 $\mu$ M(Final Conc.) |
| Probe(10 $\mu$ M)                           | 0.5 $\mu$ l                  |
| RNA Template                                | 0.01-1000ng                  |
| ROX (to be selected based on machine model) | 0.5 $\mu$ l                  |
| RNase Free Water                            | Up to 25 $\mu$ l             |

2. Recommended qPCR reaction procedure:

3. Two step amplification program:

| Cycle | Temperature | Time  |
|-------|-------------|-------|
| 1     | 50°C        | 10min |
| 1     | 95°C        | 1min  |
| 40-45 | 95°C        | 10s   |
|       | 60°C        | 20s   |

## Note

1. When using, please mix gently upside down to avoid foaming, and use after slight centrifugation;
2. Please use new (non polluting) gun heads, microtubes, etc. for the preparation and packaging of the reaction solution, and try to avoid RNase contamination as much as possible;
3. To ensure effective response, high-quality RNA templates need to be used.
4. If using a fluorescence quantitative instrument that requires ROX correction, please add the corresponding ROX according to the model used. If no correction agent is added to the system, please set the fluorescence internal reference to "None" on the instrument before conducting data analysis.