# HiScript III One Step qRT-PCR Probe 5 × Master Mix

**#Cat: NB-54-0396-01** **Size: 100rxns(20ul/rxn)**
**#Cat: NB-54-0396-02** **Size: 1000rxns(20ul/rxn)**
**#Cat: NB-54-0396-03** **Size: 10000rxns(20ul/rxn)**

## Product Description

HiScript III One Step qRT-PCR Probe 5 × Master Mix is single-tube RT-qPCR premix that is suitable for singleplex or multiplex qPCR detection using RNA (e.g. RNA virus) as template, with extremely high stability. The reaction can be performed directly after adding templates, primers and probes. Using gene specific primers (GSP), the reverse transcription and qPCR can be finished in one tube, signifi- cantly reducing pipetting procedures and the risk of contamination.

This kit can prevent the contamination of PCR product. Combining the superior performance of HiScript III Reverse Transcriptase and hot-start Champagne Taq DNA Polymerase, with an optimized buffering system, the detection sensitivity of HiScript III One Step qRT-PCR Probe 5 × Master Mix can reach 0.1 pg of total RNA or less than 10 copies of RNA templates and is suitable for high-specificity detection systems based on fluorescence labelled probes (e.g. TaqMan).

## Components

| **Components** | NB-54-0396-01<br>100 rxns (20 µl/rxn) | NB-54-0396-021,000<br>rxns (20 µl/rxn) | NB-54-0396-03<br>10,000 rxns (20 µl/rxn) |
|---|---|---|---|
| ☐ RNase-free ddH2O | 1 ml | 10 ml | 100 ml |
| ■ One Step qRT-PCR | | | |
| Probe 5 × Master Mix | 400 µl | 4 × 1 ml | 40 ml |
| 50 × ROX Reference Dye 1 | 40 µl | 400 µl | 4 × 1 ml |
| 50 × ROX Reference Dye 2 | 40 µl | 400 µl | 4 × 1 ml |

a. It contains dNTP Mix, Mg2+, HiScript Ⅲ Reverse Transcriptase, RNase inhibitor, and Champagne Taq DNA Polymerase.
b. Used to rectify the error of fluorescence signals between different wells. Use 50 × ROX Reference Dye 1 for ABI 7900HT/7300 Real-Time PCR System and StepOnePlus; Use 50 × ROX Reference Dye 2 for ABI 7500, 7500 Fast Real-Time PCR System, and Stratagene Mx3000P. Don't use ROX for Roche and Bio-Rad Real-Time PCR instruments.

**Storage**

Store at -30 ~ -15℃ and transport at ≤0℃.

**Applications**

This product is suitable for detection of various RNA nucleic acids of animals, plants and microorganisms (viruses, etc.).

**Notes**

For research use only. Not for use in diagnostic procedures.

1. One Step qRT-PCR Probe 5 × Master Mix contains high concentration of glycerol. Please centrifuge briefly and mix gently before use.
2. To avoid contamination, please use RNase-free tips and EP tubes.

**Experiment Process (Using ABI StepOnePlus)**

**1. Prepare the reaction solution in a RNase-free PCR tube as follows**

| | |
|---|---|
| RNase-free ddH2O | to 20 µl |
| One Step qRT-PCR Probe 5 × Master Mix1 ml | 4 µl |
| 50 × ROX Reference Dye 1 | 0.4 µl |
| Primer Forward (10 µM) | 0.4 µl |
| Primer Reverse (10 µM) | 0.4 µl |
| TaqMan Probe (10 µM) | 0.2 µl |
| Template RNA | Total RNA: 1 pg - 1 µg |

For each component, the recommended volume can be adjusted as follows:

Generally, the final concentration of primer should be 0.2 µM. If necessary, it can be adjusted in the range of 0.1 - 1.0 µM.

▲ The final concentration of TaqMan Probe can be adjusted between 50 - 250 nM

▲ The accuracy of template volumes have significant impacts on the qPCR results, due to the high sensitivity of qPCR. Therefore, to improve the experimental repeatability, it is recommended to dilute the template and pipet more volume (e.g. diluted to 2 - 5 µl/sample) to the reaction system.

▲ The size of the amplified products should be within the range of 80 - 200 bp.

## 2. Reaction Program

**Standard Program (for the optimal amplification sensitivity)**

| | | | | |
|---|---|---|---|---|
| Stage 1 | Reverse Transcription | Rep: 1 | 55℃[a] | 15 min |
| Stage 2 | Initial Denaturation | Rep: 1 | 95℃ | 30 sec |
| Stage 3 | Cycling Reaction | Reps: 45 | 95℃ | 10 sec |
| | | | 60℃ | 30 sec[b] |

**Fast Program (suitable for most One Step qRT-PCR applications)**

| | | | | |
|---|---|---|---|---|
| Stage 1 | Reverse Transcription | Rep: 1 | 55℃[a] | 5 min |
| Stage 2 | Initial Denaturation | Rep: 1 | 95℃ | 30 sec |
| Stage 3 | Cycling Reaction | Reps: 45 | 95℃ | 5 sec |
| | | | 60℃ | 20 sec[c] |

a. For templates with complex secondary structure or high GC content, the reverse transcription temperature can be increased to 55℃, which will improve the sensitivity and performance.

b. The extension time varies between different qPCR instruments used. For ABI 7700 and 7900HT, the extension time should be ≥30 sec; for ABI 7000 and 7300, the extension time should be ≥31 sec; and for ABI 7500, ≥34 sec;

c. Please check if the fast program is compatible with the qPCR instrument.

## 3. Data analysis of the Real Time PCR amplification curve and the standard curve, etc.