# One-Step qRT-PCR Kit

Cat. No. SM307-1000 Size: 1000 Reactions
Cat. No. SM307-0100 Size: 100 Reactions
Store at -20°C

## Description

The One-Step qRT-PCR Kit are compatible with TaqMan probes. The Kit provides a convenient, sensitive, and reproducible method to detect and quantify the RNA molecules by quantitative reverse transcription polymerase chain reaction (qRT-PCR). The components for cDNA synthesis, PCR amplification, and quantification are combined in a kit, using gene-specific primers, probes, and target RNAs from either total RNA or mRNA. The kit consists of three major components: RT/ HotStar Taq Mix, 2X Reaction Mix, and 50 mM Magnesium Sulfate ($MgSO_4$). The RT/ HotStar *Taq* Mix contain a mixture of Reverse Transcriptase (RTase) and HotStar *Taq* DNA polymerase for optimal cDNA synthesis and PCR amplification. The RTase is modified from the Moloney Murine Leukemia Virus (M-MLV) RTase, engineered to reduce RNase H activity and increase thermal stability. The HotStar Taq DNA polymerase is *Taq* DNA polymerase complexes with a proprietary antibody that blocks activity at ambient temperatures. Activity is restored after the enzyme activation step at 95°C, thereby providing an automatic "hot-start" for *Taq* DNA polymerase in PCR, increasing the sensitivity and specificity of PCR reaction. The 2X Reaction Mix consists of 6 mM $MgSO_4$, deoxyribonucleotide triphosphates (dNTPs), and stabilizers provide a proprietary buffer system optimized for reverse transcription and PCR amplification. One addition tube of 50 mM MgSO4 is included in the kit. For COVID-19 detection, the optimal concentration is 4 mM. An additional 0.5 µl for each reaction is recommended.

## Feature

- Time efficiency – included most reagents for qRT-PCR reaction.

## Applications

- Detection of expressed genes.
- Examination of transcript variants.

## Kit Contents

| Contents | SM307-1000 | SM307-0100 |
|---|---|---|
| RT/ HotStar Taq Mix | 500 µl | 50 µl |
| 2X Reaction Mix | 12.5 ml | 1.25 ml |
| 50 mM $MgSO_4$ | 1 ml x 2 vials | 200 µl |

## Quality Control

The quality of the One-Step qRT-PCR Kit is tested on a lot-to-lot basis to ensure consistent product quality.

## Required Materials

- Reaction tubes and caps /qPCR plates and seals
- qPCR thermal cycling instrument
- Gene-specific primers and fluorogenic probe
- RNase inhibitor (optional)
- qPCR thermal cycling instrument
- DEPC- treat water
- ROX reference dye (optional)

## Compatible Instruments

Depending on the use of fluorescent PCR instrument, check on the following table, optimal cycling conditions will vary with different instruments:

| Product Name | qRT-PCR Thermal Cycling Instrument |
|---|---|
| One-Step qRT-PCR Kit | Applied Biosystems™ 7000, 7300, 7500, 7700, and 7900HT Real-Time PCR Systems<br>Applied Biosystems™ GeneAmp™ 5700<br>Bio-Rad™ iCycler™<br>Agilent™ Mx3000P™, Mx3005P™, and Mx4000™<br>Corbett Research Rotor-Gene™<br>MJ Research DNA Engine Opticon™, Opticon™ 2, and Chromo4™ Real-Time Detector<br>Cepheid SmartCycler™ thermal cyclers |

## One-Step qRT-PCR Kit Protocol

1. Thaw the One-Step qRT-PCR Kit, template RNA, primers, fluorogenic probe, ROX reference dye (optional) and DEPC-treat water on ice. Mix each solution well.
2. Set up the following reaction mixture, and reaction cocktails can be made when multiple reactions are being assembled.

| Component | Volume (µl) | Final Concentration |
|---|---|---|
| 2X Reaction Mix | 12.5 µl | 1X |
| Template RNA | Variable | 1 pg-1 µg |
| Sense Primer (10 µM) | 0.5 µl | 200 nM |
| Anti-Sense Primer (10 µM) | 0.5 µl | 200 nM |
| 50 mM $MgSO_4$ (optional) | 0.5 µl | |
| RT/ HotStar *Taq* Mix | 0.5 µl | |
| Fluorogenic probe (10 µM) | 0.25 µl | 100 nM |
| ROX Reference Dye (optional) | Variable | |
| RNase Inhibitor (optional) | 0.5 µl | 20 U |
| Add DEPC treat water to | 25 µl | |

3. Gently mix and make sure that all the components are at the bottom of the amplification tube. Centrifuge briefly if needed.
4. Perform qRT-PCR reactions using the following cycling program:

| Temperature (°C) | Standard Mode Process | Fast Mode Process | Cycles |
|---|---|---|---|
| 50 | 15 minutes | 5 minutes | 1 |
| 95 | 2 minutes | 2 minutes | 1 |
| 95 | 15 seconds | 3 seconds | 40 |
| 60 | 30 seconds | 30 seconds | |

5. Collect the data and analyze results.

## Troubleshooting

Refer to the table below to troubleshoot problems that you may encounter when you did qRT-PCR amplification with the kit.

| Problem | Cause | Solution |
|---|---|---|
| No signal, or one or more signals detected late in qRT-PCR | Wrong cycling conditions | Always start with the optimized cycling conditions specified in the protocols. Be sure that the cycling conditions include the initial step for cDNA synthesis, RTase Inactivation / Hot-start *Taq* Activation, and the specified times for denaturation and annealing/extension. |
| | Wrong or no detection step | Ensure that fluorescence detection takes place during the combined annealing/extension step. |
| | Problems with starting template | Check the concentration, storage conditions, and quality of the starting template. If necessary, make new serial dilutions of template nucleic acid from the stock solutions. Repeat the qRT-PCR using the new dilutions. |

## Related Ordering Information

| Cat. No. | Description | Size |
|---|---|---|
| SN017-0100 | Total RNA Isolation Kit (Blood/ Cultured Cell/ Fungus) | 100 Reactions |
| SN020-0100 | Total RNA Isolation Kit (Plant) | 100 Reactions |
| SN021-0100 | Total RNA Isolation Kit (Tissue) | 100 Reactions |
| SR001-2500 | RiboIN RNase Inhibitor, 40 U /µl | 2500 U |

## Caution

- During operation, always wear a lab coat, disposable gloves, and protective equipment.
- Research Use Only. Not intended for any animal or human therapeutic or diagnostic uses.