SMOBIO
Small Bio, Smart Tool

**Product Information** ExcelRT™series

## ExcelRT™ One-Step RT-qPCR Kit

| | | |
|---|---|---|
| **RQ2200** | **200 RXN** | |
| | 2X One-Step Master Mix (TaqMan, **no** ROX) | 2 x 1 ml |
| | One-Step RT Enzyme Mix | 400 µl |

### Storage

**Aliquot to avoid multiple freeze-thaw cycles**
**Protect from light**
-20°C for 12 months

### Features

- High specificity
- With **no** ROX reference dye
- Suitable for fast program
- Reverse transcription at wide temperature range (42°C-60°C)

## Description

The ExcelRT™ One-Step RT-qPCR kit (TaqMan, **no** ROX) is designed for reverse transcription and quantitative real-time analysis of a specific target RNA by one-step reaction. The ExcelRT™ One-Step RT-qPCR kit (TaqMan, **no** ROX), consisting of One-Step RT Enzyme Mix and 2X One-Step Master Mix, is a convenient kit designed for highly efficient cDNA synthesis and high specific real-time PCR in a single tube. The One-Step RT Enzyme Mix contains a thermostable ExcelRT™ Reverse Transcriptase and a RNAok™ RNase inhibitor. Consequently, One-Step RT Enzyme Mix can reverse transcribe RNA to cDNA at a wide temperature range from 42 to 60°C and be active against RNase A, RNase B and RNase C. By containing specialized hot-start *Taq* DNA polymerase, which greatly reduce primer-dimer formation and can be activated within 2 minutes, the 2X One-Step Master Mix features high specificity and is suitable for fast cycle program.

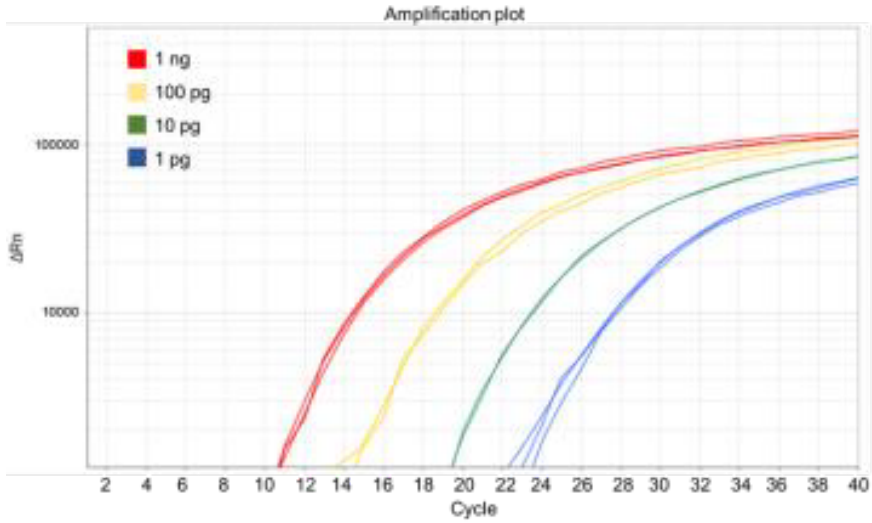


Fig. 1. ExcelRT™ One-Step RT-qPCR Kit can quantitatively analyze target RNA from a wide range of RNA template input. The amplification plot of one-step RT-qPCR with total RNA templates ranging from 1 pg to 1 ng in quantity, analyzed by using RQ2200 ExcelRT™ One-Step RT-qPCR Kit (TaqMan, **no** ROX) for RT-qPCR amplification.

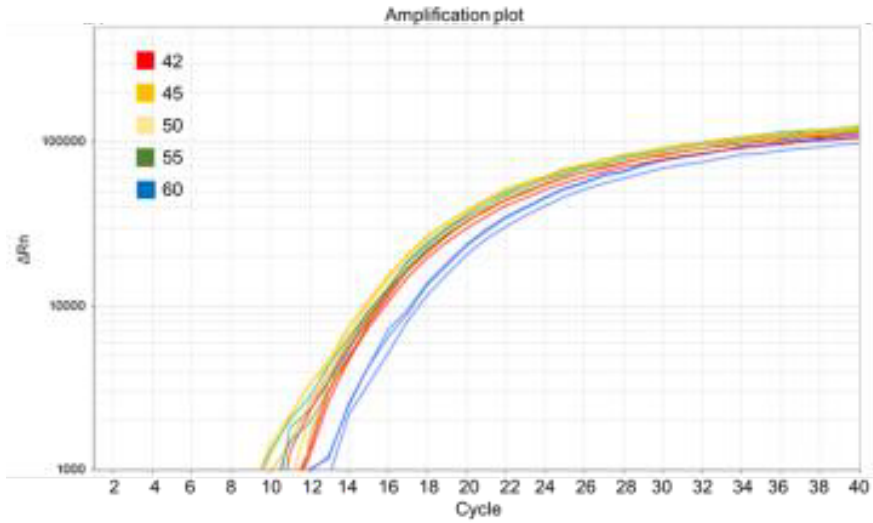


Fig. 2. ExcelRT™ One-Step RT-qPCR Kit can quantitatively analyze target RNA at a wide temperature range (42-60°C). The overlapped amplification plot of one-step RT-qPCR with reverse transcription at temperature range from 42 to 60°C, analyzed by using RQ2200 ExcelRT™ One-Step RT-qPCR Kit display that ExcelRT™ One-Step RT-qPCR Kit preforms successfully cDNA synthesis at wide temperature range.

## Instrument compatibility

- Applied Biosystems system:
  - 5700, 7300, 7000, 7700, and 7900HT system
  - StepOne™ / StepOne Plus™
  - QuantStudio™ 3 / 5/ 6 / 7
- BioRad system:
  - CFX96 / CFX384
  - Chromo 4™ Real-Time Detector
  - DNA Engine Opticon™ / Opticon™ 2
- Roche system:
  - Roche LightCycler® 480 / Nano
- Cepheid system:
  - Smart Cycler®
- Eppendorf system:
  - Mastercycler® ep realplex
- QIAGEN system:
  - Rotor-Gene™ Q

Note:

1. Selection of fluorescent reporter dye of TaqMan probe should refer to optical detection system of instruction. ExcelRT™ One-Step RT-qPCR kit (TaqMan, **no** ROX) is compatible with a variety of real-time instruments, including but not limited to the list above.

## Recommended primer design

- Amplicon size: 80-150 bp
- Tm value: around 60°C (calculated with Primer3 software)
- Primer length: 17-25 mer
- Sequence:
  - 45-55% of GC content is recommended.
  - Avoid regional high GC or AT content
  - Avoid palindrome sequence
  - Sequence with G or C at the 3' end is recommended.
- Specificity of primers should be confirmed through a BLAST search.

## Recommended probe design

- Tm value: 6-10°C higher than primers
- Probe length: 20-30 mer
- Sequence:
  - 35-65% of GC content is recommended.
  - Avoid regional high GC or AT content
  - Select the strand contains more C’s than G’s
  - Avoid palindrome sequence
  - Avoid a G at the 5' end to prevent quenching of the 5’ fluorophore.
- Specificity of probe should be confirmed through a BLAST search.

# Recommended reaction mixture set up for qPCR

| | volume | Final concentration |
|---|---|---|
| **Template RNA** | Varied | 1 pg – 1 µg |
| **Forward primer (10 µM)** | Varied | 125 – 900 nM |
| **Reverse primer (10 µM)** | Varied | 125 – 900 nM |
| **TaqMan Probe (10 µM)** | Varied | 100 – 200 nM |
| **One-Step RT Enzyme Mix** | 2 µl | 1X |
| **2X One-Step Master Mix** | 10 µl | 1X |
| **dd$H_2O$** | Up to 20 µl | - |
| **Total volume** | 20 µl | - |

*Template amount varies depending on the copy number of target present in the template solution..

** The PCR primer and probe concentration for an optimal qPCR reaction may vary according to primers' and probe's properties.

# Recommended qPCR program

# standard

| Step | Cycles | temperature | Time |
|---|---|---|---|
| **Reverse transcription** | 1 | 42˚C - 60˚C<br>( 45˚C- 55˚C is recommended) | 10 mins |
| **Enzyme activation** | 1 | 95˚C | 3 mins |
| **Denaturation** | 40-50 | 95˚C | 15 seconds |
| **Annealing/ Extension** | | 60˚C | 1 mins |

**( to be continued)**

## Fast program

| Step | Cycles | temperature | Time |
|---|---|---|---|
| **Reverse transcription** | 1 | 42˚C - 60˚C<br>( 45˚C- 55˚C is recommended) | 5 mins |
| **Enzyme activation** | 1 | 95˚C | 20 seconds |
| **Denaturation** | 40-50 | 95˚C | 3 seconds |
| **Annealing/ Extension** | | 60˚C | 30 seconds |

## Other Information

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Caution: Not intended for human or animal diagnostic or therapeutic uses.

## Related Products

| | |
|---|---|
| RP1000 | ExcelRT Reverse Transcriptase, 20,000 U |
| RP1100 | ExcelRT One-step RT-PCR Kit, 50 RXN |
| RP1400 | ExcelRT Reverse Transcription Kit II, 100 RXN |
| RI1000 | RNAok RNase Inhibitor, 2000 U |
| TQ1100 | ExcelTaq 2X Q-PCR Master Mix (SYBR, no ROX), 200 RXN |
| TQ1210 | ExcelTaq 2X Fast Q-PCR Master Mix (SYBR, ROX), 200 RXN |
| TQ2110 | ExcelTaq 2X Q-PCR Master Mix (TaqMan, ROX), 200 RXN |
| DM2300 | ExcelBand 100 bp+3K DNA Ladder, 500 µl |
| DM3100 | ExcelBand 1 KB (0.25-10 kb) DNA Ladder, 500 µl |
| DL5000 | FluoroDye DNA Fluorescent Loading Dye (Green, 6×), 1 ml |
| NS1000 | FluoroVue Nucleic Acid Gel Stain (10,000X), 500 µl |
| PM2510 | ExcelBand Enhanced 3-color Regular Range Protein Marker, 250 µl × 2 |
| TF1000 | SMO-HiFi DNA Polymerase, 100 U × 1 |
| TP1000 | ExcelTaq Taq DNA Polymerase, 500 U × 1 |
| TP1200 | ExcelTaq 5X PCR Master Dye Mix, 200 RXN |