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Product Information

ExcelRT™ series

ExcelRT™ One-Step RT-qPCR Kit (TaqMan, no ROX)

RQ2200	200 RXN	
	2X One-Step Master Mix (TaqMan, no ROX)	1 ml x 2
	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.

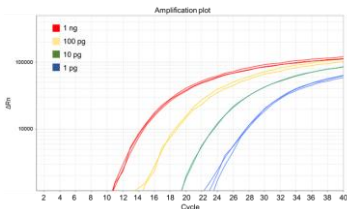


Fig. 1. ExcelRT™ One-Step RT-qPCR Kit can quantitatively analyze target RNA from a wide range of RNA template input. The amplification plot of one-step RT-qPCR with total RNA templates ranging from 1 pg to 1 ng in quantity, analyzed by using RQ2200 ExcelRT™ One-Step RT-qPCR Kit (TaqMan, **no** ROX) for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

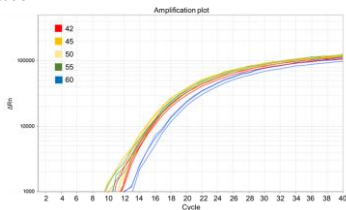


Fig. 2. ExcelRT™ One-Step RT-qPCR Kit can quantitatively analyze target RNA at a wide temperature range (42-60°C). The overlapped amplification plot of one-step RT-qPCR with reverse transcription at temperature range from 42 to 60°C, analyzed by using RQ2200 ExcelRT™ One-Step RT-qPCR Kit display that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

- T_m 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
ddH ₂ O	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 (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 (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
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
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

The latest version of the manual can be downloaded from www.smobio.com/shop.