Product Information ExcelRT™series

ExcelRT™ One-Step RT-qPCR Kit

RQ2110	200 RXN	
	2X One-Step Master Mix	
	(TaqMan, 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 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, 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, 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. This master mix includes ROX reference dye for normalization of each RT-qPCR assay.

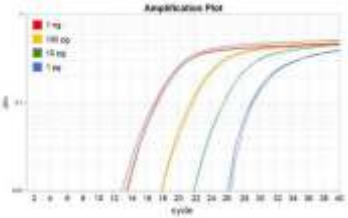

Fig. 1. ExcelRT™ One-Step RT-qPCR Kit can quantitatively analyze target RNA from a wide range of RNA template input. The amplification plot of one-step RT-qPCR with total RNA templates ranging from 1 pg to 1 ng in quantity, analyzed by using RQ2110 ExcelRT™ One-Step RT-qPCR Kit (TaqMan, ROX) for RT-qPCR amplification.

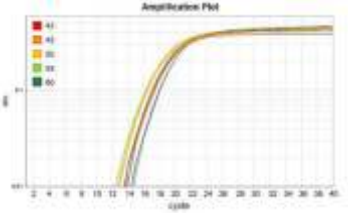

Fig. 2. ExcelRT™ One-Step RT-qPCR Kit can quantitatively analyze target RNA at a wide temperature range (42-60°C). The overlapped amplification plot of one-step RT-qPCR with reverse transcription at temperature range from 42 to 60°C, analyzed by using RQ2110 ExcelRT™ One-Step RT-qPCR Kit display that 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, ROX) is compatible with a variety of real-time instruments, including but not limited to the list above.

2. ExcelRT™ One-Step RT-qPCR kit (TaqMan, ROX) is high ROX reagent. Although it is compatible with a variety of real-time instruments, it loses the advantage of normalization provided by ROX when applicating to no ROX or low ROX real-time instruments.

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
ddH_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 (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
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