Ver.23.04

One-Step qRT-PCR Kit for probe

(w/o fluorescence dye)

Cat.No. ORQ811-R

Description: One-Step qRT-PCR Kit for probe is designed for reverse transcription and quantitative real-time analysis of a specific target RNA by one-step reaction. The kit consists of One-Step RT Enzyme Mix and 2X One-Step Master Mix. The One-Step RT Enzyme Mix contains a thermostable Reverse Transcriptase and a RNase inhibitor. 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. 2X One-Step Master Mix 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.

Product contents:

1) 2X One-Step Master Mix (TaqMan, no ROX): 500µL
2) One-Step RT Enzyme Mix: 100µL

Storage

- Store at –20°C in dark for 12 months
- Aliquot to avoid multiple freeze-thaw cycles Protect from light

Recommended primer design

- Amplicon size: 80-150bp
- Tm value: around 60°C
- Primer length: 17-25mer
- 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: 20-30mer
- 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	1pg – 1µg
Forward primer (10µM)	Varied	125-900nM
Reverse primer (10µM)	Varied	125-900nM
TaqMan Probe (10µM)	Varied	100-200nM
One-Step RT Enzyme Mix	2µL	1X
2X One-Step Master Mix	10µL	1X
Add D.W to	Up to 20µL	-

Note:

- **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 primer's and probe's properties. Recommended qPCR program**

Recommended qPCR Program

Standard program

Steps	Cycles	Temperature	Time
Reverse transcription	1	42-60°C (42-55°C is recommended)	10 min
Enzyme activation	1	95°C	3 min
Denaturation	40-50	95°C	15 sec
Annealing/Extension		60°C	1 min

Fast program

Steps	Cycles	Temperature	Time
Reverse transcription	1	42-60°C (42-55°C is recommended)	5 min
Enzyme activation	1	95°C	20 sec
Denaturation	40-50	95°C	3 sec
Annealing/Extension		60°C	30 sec

Note

- You may modify the amount of template, extension time, annealing temperature, and the number of PCR cycles according to the target size, primer's Tm, and the type of templates for amplification.