Ver.23.06

OneStep Multiplex qRT-PCR Master Mix for Probe (4X)

Cat.No. ORQ812-R

DESCRIPTION: OneStep Multiplex qRT-PCR Master Mix for Probe (4X) is used to perform one-step multiplex real-time PCR applications with any gene-specific primer and probe sets, and is suitable for both RNA and DNA targets. This master mix is formulated with optimized buffer components to accommodate multiplex amplification of up to four RNA or DNA target sequences in a single reaction.

The master mix is supplied at a 4X concentration that allows to input more sample into each reaction, increasing sensitivity even in low-volume reactions. The master mix consists of heat-resistant reverse transcriptase, hot-start *Taq* DNA polymerase, RNase inhibitor, Heat-labile UDG, dNTPs, etc., which makes the product performance more stable and can be stored for a long time.

PRODUCT CONTENTS:

- OneStep Multiplex qRT-PCR Master Mix for Probe (4X): 0.5mL
  Note: 0.5mL is sufficient for 100 reactions (Reaction volume 20µL)

STORAGE

- Store at –20°C for 12 months
- Prepare aliquots to avoid multiple freeze-thaw cycles.

PROTOCOL

Preparation of Reaction System

- Prepare the reaction system on ice by referring the table below.
- Reserve a 10% margin for pipetting loss while preparing for multiple reaction.

Fast Reaction System

Component	Volume	Final Concentration
OneStep Multiplex qRT-PCR Master Mix for Probe (4X)	5µL	1X
Primer-Probe Mix	1µL	Primer: 400 – 900nM Probe: 100 – 250nM
Sample*	Add as needed	1pg – 100ng
RT-PCR Grade Water	Add as needed	
Total Volume	20µL	

Steps	Stage	Cycles	Temperature	Time
Reverse transcription	1	1	55°C#	10 min
Polymerase activation	2	1	95°C	2 min
Amplification	3	45	95°C	3 sec
			60°C	30 sec

*** DNA or RNA samples can be used. Reverse Transcription does not affect DNA samples.**
 # The temperature can be adjusted between 48°C – 55°C

Standard Reaction System

Component	Volume	Final Concentration
OneStep Multiplex qRT-PCR Master Mix for Probe (4X)	12.5µL	1X
Primer-Probe Mix	2.5µL	Primer: 400 – 900nM Probe: 100 – 250nM
Sample*	Add as needed	1pg – 100ng
RT-PCR Grade Water	Add as needed	
Total Volume	50µL	

Steps	Stage	Cycles	Temperature	Time
Reverse transcription	1	1	55°C#	10 min
Polymerase activation	2	1	95°C	2 min
Amplification	3	45	95°C	15 sec
			60°C	60 sec

*** DNA or RNA samples can be used. Reverse Transcription does not affect DNA samples.**

 # The temperature can be adjusted between 48°C – 55°C

RESULT ANALYSIS

Data analysis varies depending on the instrument used. Please refer to your instrument user guide for information. In general, data analysis mainly includes:

1. Observe the amplification curve and set it according to needs, such as:
   - Set appropriate baselines and threshold lines.
   - Remove some typical outliners from the analysis.
2. Observe whether there is any difference in Ct value between multiple wells.
3. For absolute quantification, observe the slope, amplification efficiency, R^2 value, intercept, Ct value and outliners if the standard curve.