M-MLV III One-Step qPCR-PCR(Probe) Kit

产品信息：

组成	MT701-01 20μl×100 rnxs
2x One-Step qPCR-PCR mix(Probe)	1×1ml
M-MLV III Enzyme mix	1×60μl
DEPC-H_2O	1×1ml

Storage and stability:

Is shipped on Dry Ice and can be stored for up to 12 months at -20°C,or up to 2 weeks at 4°C. Repeated freeze/thaw cycles should be avoided.

Notes: Research only

Description：

The M-MLV III One-Step qPCR-PCR(Probe) Kit has been formulated for highly reproducible first-strand cDNA synthesis and subsequent real-time PCR in a single tube. The kit is formulated for use with probe-detection technology, including TaqMan®,Scorpions® and molecular beacon probes.A combination of the latest advances in buffer chemistry together with a reverse transcriptase and hot-start DNA polymerase system, ensures that RealFAST Probe No-ROX One-Step Kit produces fast, highly-specific and ultra-sensitive one-step RT-qPCR.

The M-MLV III One-Step qPCR-PCR(Probe) Kit consists of a 2x One-Step mix(Probe), as well as separate reverse transcriptase and RNase Inhibitor.

PCR Reaction Conditions(for a 20μl reaction)

2x One-Step qPCR-PCR mix(Probe)	10μl
10μM Forward Primer	0.8μl
10μM Reverse Primer	0.8μl
10μM Probe	0.2μl
M-MLV III Enzyme mix	0.6μl
Template and Primers	as required
ddH_2O	up to 20μl

PCR cycling conditions:

The following RT-qPCR conditions are suitable for the M-MLV III One-Step qPCR-PCR(Probe) Kit with the majority of amplicons and real-time PCR instruments. However, the cycling conditions can be varied to suit different probe-based reactions or machine-specific protocols.The detection channel on the real-time instrument should be set to acquire at the appropriate wavelength(s). We recommend using the following cycling conditions for optimal results:

Step	Temp	Time	Cycles
Reverse transcription	45°C	10min	1
Polymerase activation	95°C	2min	1
Denaturation	95°C	5s	40
Annealing/ Extension	60°C	20-30s	

RT-qPCR optimization: The following optimization may be necessary to improve the efficiency of some reactions, such as multiplexing with more than 2 probes, or if the target amplicon is longer than 200bp.

• The reverse transcription reaction time can be extended up to 20 minutes and/or the temperature can be increased up to 48°C

• The annealing/extension time can be extended up to 60seconds and/or the temperature can be increased up to 65°C

The conditions above are intended as a guide only; conditions will vary from reaction to reaction and may need optimization.

Important considerations and optimization

Primers and probe: These guidelines refer to the use of dual-labeled probes. Please refer to the relevant literature when using other probe types. The sequence and concentration of the probe and primers, as well as amplicon length, can be critical for specific amplification, yield and overall efficiency of any RT-qPCR. We strongly recommend taking the following points into consideration when designing and running your RT-qPCR:

- use primer-design software, such as Primer3 (http://frodo.wi.mit.edu/primer3/) or visual OMPTM (http://dnasoftware.com/). Primers should have a melting temperature (Tm) of approximately 60°C. The Tm of the probe should be approximately 10°C higher than that of the primers
- optimal amplicon length should be 80-200bp, and should not exceed 400bp
- final primer concentration of 400nM is suitable for most Probe reactions, however to determine the optimal concentration we recommend titrating in the range 0.2-1μM
- use an equimolar primer concentration
- a final probe concentration of 100nM is suitable for most applications. We recommend that the final probe concentration is at least 2-fold lower than the primer concentration

Note: *In multiplex RT-qPCR, probe concentrations in excess of 100nM can result in cross-channel fluorescence*

- when possible, use intron-spanning primers to avoid amplification from genomic DNA

Template: It is important that the RNA template is intact and devoid of DNA or contaminating inhibitors of both reverse transcription and PCR. The recommended amount of template for one-step RT-qPCR is dependent upon the type of RNA used.

- **total RNA:** purified total RNA can be used in the range from 1pg to 1μg per 20μl reaction
- **mRNA:** purified mRNA can be used from 0.01pg per 20μl reaction

$MgCl_2$: The $MgCl_2$ concentration in the 1x reaction mix is 3mM. In the majority of real-time PCR conditions this is optimal for both the reverse transcriptase and the hot-start DNA polymerase. If necessary, we suggest titrating the $MgCl_2$ to a maximum of 5mM.

RT-PCR controls:

It is important to detect the presence of contaminating DNA that may affect the reliability of the data. Always include a no-RT control, by omitting the reverse transcriptase from the reaction.

Instrument compatibility

The M-MLV III One-Step qPCR-PCR(Probe) Kit has been optimized for use with all probe chemistries, including TaqMan, FRET,Scorpions and molecular beacon probes.

The M-MLV III One-Step qPCR-PCR(Probe) Kit can be used on all real-time PCR instruments.

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