

EffiStart™ 2X One-Step Probe RT-qPCR Master Mix

Cat no. LDG0033RF

Product Overview

Package component

Item	Content
2X One-Step Probe RT-qPCR Master Mix	1 vial (1 mL)

Description

EffiStart™ 2X One-Step Probe RT-qPCR Master Mix is a one-step real-time reverse transcription-polymerase chain reaction (RT-qPCR) kit developed for cDNA synthesis and real-time PCR in the same tube. This product contains Hot Start Taq DNA Polymerase (LDG0002RF) and is suitable for probe-based detection and formulated as a 2-fold premix. Reaction can be simply set up by adding the RNA template, primers, and probes. This master mix does not contain ROX reference dye; it offers great convenience and minimizes the risk of cross-contamination.

Storage and Stability

Stored at -20°C. Avoid repeated freeze/thaw cycles.

Procedure

The following procedure is a general guideline for One-step RT-qPCR reaction. To maintain an RNase-free environment, always wear disposable gloves, and use laboratory consumables and water of nuclease-free grade during the whole experiment course.

RT-qPCR reaction set-up:

- Place all required reagents on ice.

Component	Amount	Final concentration
2X One-Step Probe RT-qPCR Master Mix	10 µL	1X

Forward primer (10 µM)	0.8 µL	0.4 µM
Reverse primer (10 µM)	0.8 µL	0.4 µM
Probe (10 µM)	0.4 µL	0.2 µM
RNA template	X µL	≤ 1 µg (total RNA)
Nuclease-Free H ₂ O	Y µL	-
Total reaction volume	20 L	-

- Gently mix the reaction thoroughly to achieve uniform distribution and briefly centrifuge.
- Thermal cycling conditions for standard qPCR

Step	Cycles	Temperature	Time
Reverse transcription	1	50°C	10-15 min
Enzyme activation	1	95°C	5 min
Denaturation	40-45	95°C	5-15 sec
Annealing/Extension		55 – 65 °C	30-60 sec

Important notes

(1) Primer/Probe concentration

Final concentrations of 400 nM (each primer) are suitable for most reactions. To obtain optimal condition, primer concentration can be titrated between 0.2-1 µM.

A final concentration of 200 nM (probe) is suitable for most reactions. To obtain optimal condition, probe concentration can be titrated between 0.1-0.3 µM.

(2) Annealing/Extension optimization

To obtain optimal condition, annealing/extension temperature can be adjusted between 55°C-65°C, annealing/extension time can be extended up to 60 sec.

(3) Target length

Appropriate amplicon length should be arranged between 80-200 bp.

Disclaimer

This product is for research use only and is not intended for diagnostic use.

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