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Product Information & Manual

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EffiStart™ 5X One-Step Probe RT-qPCR Master Mix (Glycerol-Free)

Cat no. LDG0036RF

Product Overview

Package component

Item	Content
5X One-Step Probe RT-qPCR Master Mix (Glycerol-Free)	1 vial (1 mL)

Description

EffiStart™ 5X One-Step Probe RT-qPCR Master Mix (Glycerol-Free) 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 (LDG0010RF) and is suitable for probe-based detection and formulated as a 5-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. This product is a glycerol-free formulation.

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:

1. Place all required reagents **on ice**.

Component	Amount	Final concentration
5X One-Step Probe RT-qPCR Master Mix (Glycerol-Free)	4 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_2O	Y µL	-
Total reaction volume	20µL	-

2. Gently mix the reaction thoroughly to achieve uniform distribution and briefly centrifuge.
3. 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

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

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