



Product Information & Manual

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EffiStart™ 2X Probe qPCR Master Mix

Cat no. LDG0029RF

Product Overview

Package component

Item	Content
2X Probe qPCR Master Mix	1 vial (1 mL)

Description

EffiStart™ 2X Probe qPCR Master Mix is an optimized reaction mix for real-time polymerase chain reaction (qPCR) kit. 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 DNA 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 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.

qPCR reaction set-up:

1. Place all required reagents on ice.

Component	Amount	Final concentration
2X Probe 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
DNA template	X µL	≤ 250 ng
Nuclease-Free H ₂ O	Y µL	-
Total reaction volume	20 µL	-

2. Gently mix the reaction thoroughly to achieve uniform distribution and briefly centrifuge (2,500-3,000 rpm).
3. Thermal cycling conditions for standard qPCR

Step	Cycles	Temperature	Time
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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