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LEADGENE BIOMEDICAL, INC.

Product Information & Manual

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EffiStart™ 2X Probe qPCR Master Mix (Glycerol-Free)

Cat no. LDG0031RF

Product Overview

Package component

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

Description

EffiStart™ 2X Probe qPCR Master Mix (Glycerol-Free) is an optimized reaction mix for real-time polymerase chain reaction (qPCR) kit. This product contains Hot Start Taq DNA Polymerase (LDG0010RF) 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. 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 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 (Glycerol-Free)	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_2O	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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