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LEADGENE BIOMEDICAL, INC.

Product Information & Manual

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LEADSPHERE® One-Step RT-PCR Master Mix

Cat no. LDG0027RF

Product Overview

Package component

Item	Content
LEADSPHERE® One-Step RT-PCR Master Mix	Inquiry

Description

Essential components for reliable RT-PCR are included in each single LEADSPHERE® One-Step RT-PCR Master Mix, which saves time for reaction set-up and reduces the risk of contamination and pipetting errors. In addition, the stabilized format facilitates shipment and storage without cooling. LEADSPHERE® One-Step RT-PCR Master Mix includes reverse transcriptase, DNA polymerase, high-quality dNTPs, and optimized PCR buffer. This product is suitable for probe-based detection and endpoint DNA amplification. The reaction can be set up by reconstituting the LEADSPHERE™ One-Step RT-PCR Master Mix by adding PCR-grade water along with the RNA template, primers, and probe to a total volume of 20 µL.

Storage and Stability

Stored at 4-30°C and keep dry.

Procedure

The following procedure is a general guideline for a 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
LEADSPHERE® One-Step RT-PCR Master Mix	1 tablet	1 X
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	20 µL	-

2. Prepare the above reagents and gently mix to 20 µL total reaction volume 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	40-50	95°C	5 – 15 sec
Annealing/Extension		55-65°C	30 – 60 sec

Important notes

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.

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.

Target length

Appropriate amplicon length should be arranged between 80-200 bp

LEADGENE
www.leadgenebio.com
TEL: +886-6-253-6677 • Email: info@leadgene.com.tw • No.9, Ln. 147, Zhengbei 1st Rd., Yongkang Dist., Tainan City 710, Taiwan

Disclaimer

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

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No.9, Ln. 147, Zhengbei 1st Rd., Yongkang Dist., Tainan City 710, Taiwan R.O.C. TEL: +886-6-2536677 FAX: +886-6-2531536

www.leadgenebio.com