Data sheet

CustomProbe 2X qPCR Master Mix

Catalog Number: E0339 (ROX) Catalog Number: E0340 (ROX)

Introduction

CustomProbe 2X qPCR Master Mix has been formulated specifically for TagMan[®] probe-based real-time PCR analysis of DNA samples.

Based on the TaqMan[®] probe detection principle, the 5'-3'-quencher reporter dve and dual-labelled oligonucleotide hybridizes on a specific region within the amplified fragment. During amplification, the probe is cleaved and the reporter dye (fluorophore) is released. The fluorescent signal intensity detected is proportional to the number of amplicons. The Ct value is used for quantification purposes.

Available with the option of ROX[™] as the internal passive reference dye. The kit contains all reagents required for real-time PCR in a premixed 2x concentrated ready-to-use solution (with the exception of primer and template DNA) to ensure fast and easy preparation with a minimum of pipetting steps.

Features

- ready-to-use Master Mix
- Allow accurate quantification of a variety of gene targets
- Reduce pipetting steps to minimize the risk of • contamination
- ROX^{TM} as reference dye (1x concentrated)

KIT CONTENTS

Cat.	Quantity	
E0339*	2 x 1.25 mL	
E0340*	4 x 1.25 mL	

*Contains 2 tubes (1.25 ml) sufficient for 250 reactions

Storage:

CustomProbe 2X qPCR Master Mix is shipped on dry/blue ice. All kit components should be stored at -20°C upon receipt.

ROX[™] reference dye is sensitive to exposure to light. Avoid repeated freezing and thawing. Always ensure that the product has been fully thawed and mixed before use.

Quality Control:

Functionally tested in Real Time PCR.

Instrument Compatibility: Applied Biosystems: ABI 7500 and ABI 7500 Fast, ABI ViiA7. Stratagene(Agilent): Mx3000[™], Mx3005P[™], and Mx4000[™], Mx4000R. Other instruments that do not require the use of a passive reference.

Applications:

- Detection and quantification of DNA and cDNA targets
- Profiling gene expression
- Microbial detection
- Virus detection and quantification
- SNP genotyping assays
- High throughput applications

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BASIC REACTION CONDITIONS FOR REAL TIME PCR AMPLIFICATIONS

1. Thaw CustomProbe qPCR Master Mix (2X), template DNA, primers, probes and nuclease-free H₂O on ice. Mix each solution well.

The following protocol is recommended for a 20 μ l reaction volume:

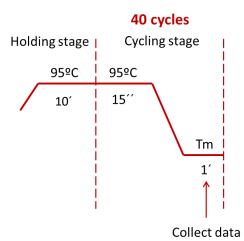
2. Set up the following reaction mixture

Component	Volume reaction 20 µL	Final concentration
CustomProbe qPCR Master Mix (2X)	10 µL	1X
Forward Primer	Χ μL	100-800 nM ⁽¹⁾
Reverse Primer	XμL	100-800 nM ⁽¹⁾
Template DNA	X μL	≤10 ng/reaction ⁽²⁾
TaqMan Probe	X μL	100 - 300 nM
Nuclease-Free Water to a final volume of	20 µL	

 $^{(1)}$ The recommendation for final primer concentration is 0.5 μ M but it can be varied in a range of 0.1-0.8 μ M if needed. ⁽²⁾For gDNA used 100-300 ng DNA.

3. Mix reagents completely, and then transfer to a thermocycler.

4. Perform the following cycling conditions (Standard):



As with all Real Time PCR reactions, conditions may need to be optimized. You may be able to adjust your PCR conditions to optimize reaction.

PRODUCT USE LIMITATION

This product is developed, designed and sold exclusively for research purposes and in vitro use only. The product was not tested for use in diagnostics or for drug development, nor is it suitable for administration to humans or animals. Please refer to www.canvaxbiotech.com for Material Safety Data Sheet of the product.

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