PR1MA™ qMAX Probe One-Step RT-qPCR Kit

Description

The qMAX Probe One-Step RT-qPCR Kit is a ready-to-use 2x master mix and companion thermostable reverse transcriptase for use in highly sensitive real-time RT-PCR assays and has been formulated for probe-detection technology, including TaqMan®, Scorpions® and molecular beacon probes. The mix is powered by HS Taq DNA Polymerase, qMAX MMLV derived Reverse Transcriptase, and an optimized buffer chemistry for robust first-strand cDNA synthesis and real-time PCR in a single tube. The mix delivers earlier quantification cycle values (Ct) and broad range detection for increased sensitivity, speed, reliability and reproducibility. The qMAX Probe One-Step RT-qPCR Kit can be used to quantify a specific target RNA from either total RNA or mRNA while reducing the number of pipetting steps and time to result.

Upon receipt, immediately store at -20°C. Avoid excessive freeze/thaw cycles. When stored as directed, this product will retain its activity for 12 months from date of receipt. The product may also be stored at 4°C for up to one month.

Limitations of Use

For research purposes only. Not intended for therapeutic or diagnostic use.

Quality Control

PR1MA enzymes and reagents are tested under general assay conditions for activity, reproducibility, efficiency, heat activation, sensitivity, and absence of nuclease contamination and nuclease activity. This product is manufactured under a comprehensive quality management system, following ISO 9001:2008 standards.

MidSci is not responsible for consequential or incidental damages, direct or indirect, resulting from use of this product. MidSci guarantees the performance of this product as described when used in accordance with these instructions.

General Guidelines

Reaction Mix

The reaction mix has been optimized to perform with maximum sensitivity and efficiency. The use of additives is not required or recommended.

20X Thermostable RTase: The 20X Thermostable Reverse Transcriptase is blended with a potent RNase Inhibitor.

Template: Use 10pg to 100ng total RNA per reaction (or a minimum of 0.01pg mRNA per reaction).

Reverse Transcription: Recommended incubation is 45°C for 10 minutes. For regions of high secondary structure, incubation temperatures up to 55°C may be used.

Primers: Primers should have a predicted melting temperature of approximately 60°C, using default Primer 3 settings (http://frodo.wi.mit.edu/primer3/). The Tm of the probe should be approximately 6°C - 10°C higher than that of the primers. For Taqman™ probes, choose a probe close to the 5' primer and avoid terminal guanosine residues.

Amplicon: Optimal extension is achieved at 72°C. The optimal amplicon length should be 80bp to 200bp and should not exceed 400bp.

When comparing qMAX Probe One-Step qPCR Mix with a reagent from an alternative supplier, we strongly recommend amplifying from a 10-fold template dilution series. Loss of detection at low template concentration is the only direct measurement of sensitivity. An early Ct value is not an indication of good sensitivity, but rather an indication of reaction speed.

Technical Support

For trouble-shooting and tech support, contact us at tech@midsci.com or call 800 227-9997.

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Reaction Setup

Thaw the qMax Probe One-Step Kit and vortex briefly. Set up reaction as follows:

Component	20µl reaction	Final concentration/Notes
2X qMax Probe One-Step Mix	10µl	1X
Forward Primer (10µM)	0.8µl	400 nM
Reverse Primer (10µM)	0.8µl	400 nM
Probe (10µM)	1.0 - 2.0 µL	1x or 2x
20X RTase Blend	1.0µl	1X Add prior to RNA
Template RNA	10pg to 100ng Total RNA	>0.01pg mRNA
PCR grade water	to final reaction volume	

For other volumes, adjust the amount of each component accordingly.

Protocol

Program the qPCR instrument using the following conditions, acquiring data, on the appropriate channel:

1. 1 cycle, 45°C for 10 minutes. For RNA with a high degree of secondary structure, use 55°C.
2. 1 cycle, 95°C for 2 minutes for an initial denaturation and polymerase activation.
3. Perform 30-40 cycles of : 95°, 5 seconds (denaturation)
   60° - 65°C, 20-30 seconds (do not exceed 30 seconds and do not use temperatures below 60°C).

MidSci offers a full line of PCR enzymes and master mixes. Visit www.midsci. com for details.

Package contents and reordering

PR1MA qMax Probe One-Step qPCR Kit, No ROX, 100 reactions - Catalog number PR2121-N-100
PR1MA qMax Probe One-Step qPCR Kit, Low ROX, 100 reactions- Catalog number PR2121-L-100
PR1MA qMax Probe One-Step qPCR Kit, High ROX, 100 reactions- Catalog number PR2121-H-100
Includes 1ml reaction mix, 100µl RTase.

PR1MA qMax Probe One-Step qPCR Kit, No ROX, 500 reactions- Catalog number PR2121-N-500
PR1MA qMax Probe One-Step qPCR Kit, Low ROX, 500 reactions- Catalog number PR2121-L-500
PR1MA qMax Probe One-Step qPCR Kit, High ROX, 500 reactions- Catalog number PR2121-H-500
Includes 5x1ml reaction mix, 5x100µl RTase.

PR1MA qMax Probe One-Step qPCR Kit, No ROX, 1000 reactions- Catalog number PR2121-N-1000
PR1MA qMax Probe One-Step qPCR Kit, Low ROX, 1000 reactions- Catalog number PR2121-L-1000
PR1MA qMax Probe One-Step qPCR Kit, High ROX, 1000 reactions- Catalog number PR2121-H-1000
Includes 10x1ml reaction mix, 10x100µl RTase.

MIDSCI™

PR1MA™

qMax Probe One-Step RT-qPCR Kit
PR2121

Reverse Transcriptase and Buffer
100 Reaction Package contains:
1ml of 2X qMax One Step Mix and
100µl of 20X RTase Blend
100 reactions, Based on 20µl total reaction volume
Store at -20°C upon receipt

888-227-9997 custserv@midsci.com