PR1MA™ One Step RT-PCR Kit

Description

The PR1MA One-Step RT-PCR Kit has been formulated for cDNA synthesis and subsequent PCR in a single tube for end-point analysis. The RT-PCR Kit consists of MMLV derived Thermostable Reverse Transcriptase, a potent RNase Inhibitor and PR1MA HS Taq for ultra-sensitive one-step RT-PCR from as little as 1pg total RNA starting material. A highly optimized buffer chemistry allows for efficient reverse transcription and PCR of problematic sequences with significant secondary structure (GC-rich targets). The PR1MA One-Step RT-PCR Kit is ideal for determining the presence or absence of RNA templates and quantifying expression through qualitative analysis of RNA transcription levels.

- Sensitive: Optimized chemistry for detection of low-copy transcripts
- Robust: Overcomes secondary structure in problematic GC-rich targets
- Convenient: First strand full-length cDNA synthesis and PCR in a single tube

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.

General Guidelines

2X PR1MA One-Step Mix: The 2X Mix is comprised of HS Taq DNA Polymerase, 2mM dNTPs, 6mM MgCl2, and PCR enhancers for maximum efficiency, sensitivity and success with difficult amplicons. Use of additional PCR enhancer is not recommended.

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

Template: Use 1pg to 1µg 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. For amplicons above 1kb, increase incubation time to 20 minutes.

Primers: Primers should have a predicted melting temperature of around 60°C, using default Primer 3 settings (http://frodo.wi.mit.edu/primer3/). The final primer concentration in the reaction should be between 0.2µM and 0.6µM.

Annealing: A temperature gradient can be performed to determine the optimal annealing temperature. Alternatively, 60°C can be used as a starting point. For optimization, increase in 2°C increments. For example, if non-specific products are present or smearing is visible, a higher annealing temperature is required.

Extension: Optimal extension is achieved at 72°C. The optimal extension time is dependent on amplicon length and complexity.

15 seconds per kilobase(Kb) is recommended for amplification from eukaryotic genomic DNA or cDNA up to 3kb.

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 One-Step Mix and vortex briefly. Set up reaction as follows:

Component	50 µl reaction	Final concentration/Notes
PR1MA 2X One-Step Mix	25µl	1X
Forward Primer (10µM)	2µl	400 nM
Reverse Primer	2µl	400 nM
20X RTase Blend	2.5µl	1X
Template RNA	1pg to 1µg Total RNA	>0.01pg mRNA
PCR grade water	to final reaction volume	

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

Protocol

1. Incubate tube from above at 45°C for 10 minutes. For RNA with a high degree of secondary structure, incubate at 55°C.
2. Incubate at 95°C for 2 minutes for an initial denaturation and polymerase activation.
3. Perform 30-40 cycles of : 95°C, 10 seconds (denaturation)
   60° - 67°C, 10 seconds (annealing temp determined by user)
   72°C, 30-60 seconds (extension)

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

Package contents and reordering

The PR1MA One-Step RT-PCR Kit is available in 50 and 100 reaction packages. Kit includes 2X PR1MA One-Step Mix and 20X Thermostable RTase blend.

PR1MA One-Step RT-PCR Kit, 50 reactions
Catalog number PR1100-50
Includes 1.25ml of 2X One-Step Mix and 100µl of 20X RTase blend.

PR1MA One-Step RT-PCR Kit, 100 reactions
Catalog number PR1100-100
Includes 2.5ml of 2X One-Step Mix in 1.25ml aliquots and 200µl of 20X RTase blend in 100µl aliquots.

MIDSCI™

PR1MA™

One-Step
RT-PCR
Kit
PR1100

One Step Reaction Mix and RTase Blend
50 Reaction Package Contains:
1.25ml of 2X One-Step Reaction Mix and
100µl of 20X RTase Blend
50 reactions, Based on 50µl total reaction volume
Store at -20°C upon receipt

888-227-9997 custserv@midsci.com