MCLAB
Molecular Cloning Laboratories

User Manual

Version 3.1
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Product name: HoTaq One-step Real-time RT-PCR Kit

Cat #: HTRT-100, HTRT-200

Description:

RT-PCR is widely used for measuring gene expression in tissue samples or cell culture systems. Traditionally, it is performed in separated two reaction steps. First-strand cDNA is reverse-transcribed from total RNA or poly (A)+ RNA using a reverse transcriptase. After then, the cDNA is amplified by PCR using a DNA polymerase in another reaction.

MCLAB the HoTaq One-step Real-time RT-PCR Kit offers a unique system for performing probe based real-time RT-PCR in a single step within a single tube with optimized reaction condition, which utilizes our own proprietary engineered highly purified QuantumScript™ HD reverse transcriptase and hot-start Taq DNA Polymerase. No additional reagents or steps are required once the reaction is initiated. This novel kit allows you to quantitatively detect specific RNA targets with high sensitivity, unparalleled convenience and wide dynamic range.

The technique reduces the risk of cross-contamination and minimizes reagents handling steps. This method is particularly useful for applications in which the expression of a small number of genes must be analyzed in many different total RNA samples, and robust amplifies high-abundance transcripts from crude total RNA preparations.

List of Components:

Sufficient reagents are supplied in the MCLAB HoTaq One-step Real-time RT-PCR Kit for 40 or 200 rxns. Upon receipt of the kit, immediately store the components at –20 °C in a freezer without a defrost cycle. It is recommended to prevent light exposure as little as possible.
40 rxns:
2x RT-PCR Buffer*: 1.5ml x 1
25x RT-PCR Enzyme Mix: 40 ul x1
200 rxns:
2x RT-PCR Buffer*: 1.5ml x 1
25x RT-PCR Enzyme Mix: 200 ul x1
*Contains regular level of ROX, dNTPs and optimized buffer components for Real-time PCR machines ABI 7000, 7300, 7700, 7900 and Stratagene Mx 3000P, Mx 3005P.

Additional Materials Required:

The following reagents, instruments and consumables are supplied by the user:
• Template RNA

- Gene-specific primers and probe
- DEPC-treated water
- Microcentrifuge
- Real-time thermal cycler
- PCR tubes/plates

One-step Real-time RT-PCR Procedure:

1. Set up one-step real-time RT-PCR reaction in a PCR tube on ice as below:

Component	Volume	Note
RT-PCR Buffer	12.5 µL	
RT-PCR Enzyme Mix	1 µL	
Forward Primer	x	Final 50 to 900 nM*
Reverse Primer	x	Final 50 to 900 nM*
Probe	x	Final 50 to 250 nM*
RNA Template	x	10 pg to 1ug total RNA
Nuclease-free water	To 25 µL	

* The concentration might need optimization for your specific targets.
For multiple reactions, master mix should be made with 5% extra reagents to reduce pipette error.
2. Gently mix thoroughly and then centrifuge briefly.
3. Place the tube or plate in the thermal cycler and set up program using associated software. Basic cycling conditions are as following:
One cycle at 50°C for 10 to 30 minutes *;
One cycle at 95°C for 10 minutes;
Followed by 40 cycles of: 95°C for 15 seconds, 60°C for 1 minute #;
4°C hold (optional)
* Reaction time could be adjusted according RNA input.
# Cycle number and annealing temperature are experiment dependent.

Troubleshooting:

Unexpected high or none Cq (quantification cycle) value
- Your RNA may be degraded. RNA integrity should be evaluated prior to the reaction.
- Your RNA sample might be contaminated with interferers for RT-PCR reaction. RNA purity should be evaluated prior to the reaction. Sample from crude total RNA preparation should be serial diluted as input and run multiple reactions. Re-precipitate or re-isolate RNA from the source if necessary.
- Poor primers and probe design. Be sure to follow guidelines when designing primers and probe for your target gene.
- Target gene may be at very weak expression level in your sample. Paralleled control gene amplification will be helpful for troubleshooting.

Reference:

1. Higuchi R, et al (1992). BioTechnology 10:413
2. Higuchi R, et al (1993). BioTechnology 11:1026