Lyophilization One Step RT-qPCR Probe Kit

Cat. No. : RK20415

Product components

Components	Component number	Size-1 100 RXN (25 μL/RXN)	Size-2 500 RXN (25 μL/RXN)
Lyophilization RT Enzyme Mix	RM21489	10 μL	50 μL
2X Lyophilization One Step RT-qPCR Probe Mix	RM21490	1.25 mL	1.25 mL*5

Product Description

The Lyophilization One-Step RT-qPCR Probe Kit is a universal lyophilized reagent box designed for RT-qPCR reactions using probes. It includes reaction buffer, hot-start Taq polymerase, novel reverse transcriptase ABScript III Reverse Transcriptase, heat-labile UDG, and RNase inhibitor (RI). This kit utilizes RNA as a template and employs gene-specific primers. Reverse transcription and PCR reactions can be performed consecutively in the same tube, eliminating the need for additional tube opening or pipetting steps, significantly enhancing detection throughput. The kit utilizes dUTP/UDG for contamination prevention, with the heat-labile UDG quickly degrading U-containing contaminants at room temperature. The heat-labile UDG is rapidly deactivated at 50°C during reverse transcription, ensuring it does not affect the efficiency and sensitivity of RT-qPCR. This product contains glycerol content below 0.2% and includes lyoprotectants and excipients. It is suitable for lyophilization of freeze-dried powder and freeze-dried microspheres. After freeze-drying into microspheres, it can be stored at 45°C for 6 months without degradation in performance.

Storage

-20℃

Notes

1. When using 2X Lyophilization One-Step RT-qPCR Probe Mix, ensure thorough dissolution and mixing before use, and avoid direct exposure to strong light. Avoid repeated freeze-thaw cycles during use, limiting them to fewer than 5 cycles, and consider aliquoting for storage. When preparing multiple One-Step RT-qPCR reactions simultaneously, it's recommended to mix the various reagents first and then aliquot them into each reaction tube to minimize reagent loss.
2. Before dispensing the Lyophilization RT Enzyme Mix from the kit, briefly centrifuge it to collect at the bottom of the tube, then use it promptly. After use, return it immediately to the -20°C freezer for storage.
3. Use uncontaminated pipette tips and microtubes for preparing and aliquoting reaction mixtures to minimize contamination.
4. To ensure successful reactions, high-quality RNA templates are recommended.
5. This kit is compatible only with specific primers and cannot be used with random primers or Oligo dT primers for reverse transcription.
6. For one-step RT-qPCR experiments, primer lengths for amplification are recommended to be between 70-200 bp for optimal results.

Operation Description

Experimental Preparation

1. RNase-free 1.5 mL EP tubes, RNase-free PCR tubes, pipettes and tips, icebox or ice.
2. PCR probes, primers, and RNA templates.
3. Fluorescent quantitative PCR-specific tubes or plates.

Experimental Procedure

Please follow the experimental procedures as specified in the user manual of the respective brand of fluorescent quantitative PCR instrument.

1. Preparation of Lyophilized System

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1.1 Prepare the reaction mix on ice. For a 25 μL system, the following components are suggested:

Reagents	Amount (25 μL)
2X Lyophilization One Step RT-qPCR Probe Mix	12.5 μL
Lyophilization RT Enzyme Mix	0.1 μL
Nuclease-free H2O	to 25 μL

Note: If lyophilizing without templates, primers, and probes, add nuclease-free water to a total volume of 25 μL. If lyophilizing with primers and probes, supplement templates, primers, probes, and nuclease-free water to a total volume of 25 μL.

1.2 Immediately aliquot reaction mix into reaction tubes and transfer to a freeze-dryer for suitable drying cycles. To ensure long-term stability at room temperature, the lyophilized product should be packaged in a low relative humidity environment after lyophilization.

1.3 Recommended Lyophilization Parameters

Phase		Temperature (℃)	Ramp Time (min)	Hold Time (hr)	Vacuum Control (mbar)
Pre-freeze	1	-45	60	2	/
	2	-42	30	/	/
Primary Drying	3	/	/	1	0.16
	4	-42	/	8	0.16
	5	-30	1	/	0.16
	6	25	160	1.5	0.1
Secondary Drying	7	35	20	3	0.1
	8	25	20	2	0.1

2. Reagent Preparation

2.1 If the reaction mix contains templates, primers, and probes: simply add 25 μL of nuclease-free water for reconstitution.

2.2 If the reaction mix does not contain templates, primers, and probes: add 25 μL of a mixture containing templates, primers, probes, and nuclease-free water to the reaction tube containing the lyophilized RT-qPCR mixture (from step 1.1) for reconstitution.

2.3 Recommended template and primer-probe mixture system, using a 25 μL system as an example:

Reagents	Amount (25 μL)
Forward Primer (10 μM) *	0.5 μL
Reverse Primer (10 μM) *	0.5 μL
TaqMan Probe (10 μM) **	0.5 μL
Totol RNA***	2.5 μL
Nuclease-free H2O	to 25 μL

*** Note: Typically, a final primer concentration of 0.2 μM yields optimal results. If reaction performance is suboptimal, primer concentrations can be adjusted within the range of 0.1-1.0 μM. It is recommended to select amplification product lengths within the range of 70-200 bp.**

**** Note: Probe concentrations can be adjusted within the range of 50-250 nM.**

***** Note: It is recommended to use 25 pg-100 ng of Total RNA as a template in a 25 μL reaction mix volume.**

Step	Temperature	Time	Cycles
UDG Reaction	25℃	5 min	1
Reverse Transcription	50℃	5 min	1
Pre-denaturation	95℃	3 min	1
Cycle Reaction	95℃	5-15 s	40~45
	60℃	30-34 s*	

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***Note: Adjust the extension time according to the shortest data acquisition time required by your Real Time PCR instrument: Set to 30 s for StepOne Plus; 31 s for 7300; 34 s for 7500.**

2.4 After the reaction, confirm the Real Time PCR amplification curve and proceed with standard curve construction, etc.

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