GO-Lyo U+ One Step RT-qPCR Probe Kit

QL229

Version 24.2

Product Description

GO-Lyo U+ One Step RT-qPCR Probe Kit is a glycerol-free one-step RT-qPCR reagent containing a lyophilization excipient for the detection of RNA templates, which can be lyophilized with a suitable lyophilization process. This product has been optimized for functional proteins, matched screening of excipients and lyophilization process, its yield rate is improved, and its storage stability reaches the same as that of glycerol-containing products, allowing for long-term stable storage after freeze-drying. In addition, it maintains the advantages of good multiplex amplification performance, high specificity and high sensitivity. We provide complete lyophilization solution that helps to reduce the time needed to optimize the lyophilization processes and ensure lyophilization formation.

Components

	Components	QL229-01 (200 rxns)	QL229-02 (1,000 rxns)	QL229-03 (5,000 rxns)
☐	RNase-free ddH_2O	3 × 1 ml	15 ml	75 ml
■	5 × One Step Mix[a]	800 µl	4 ml	20 ml
■	One Step Lyo-Enzyme Mix[b]	200 µl	1 ml	5 ml
■	3 × Excipient[c]	1.2 ml	6 ml	30 ml
	50 × ROX Reference Dye 1[d]	80 µl	400 µl	2 × 1 ml
	50 × ROX Reference Dye 2[d]	80 µl	400 µl	2 × 1 ml

a. It includes dNTP/dUTP Mix, Mg^{2+}, etc.

b. It includes Reverse Transcriptase, RNase Inhibitor, Heat-labile UDG, and *Taq* DNA Polymerase.

c. It is used to protect enzyme activity during the freeze-drying process, maintain lyophilized product morphology and long-term storage stability.

d. It is used to correct the error of fluorescence signals between wells. 50 × ROX Reference Dye 1: ABI 7900HT/7300 Real-Time PCR System and StepOnePlus; 50 × ROX Reference Dye 2: ABI 7500, 7500 Fast Real-Time PCR System, Stratagene Mx3000P; No ROX: qPCR systems from Roche and Bio-Rad.

Storage

Store at -30 ~ -15°C and ship at ≤0°C.

Applications

It is applicable for various RNA nucleic acid detection of animals, plants, and microorganisms (viruses, etc.).

Notes

1. If ions are found to precipitate after the reagent is thawed, please leave it at room temperature for a while or mix by vortexing, and use it after dissolution.
2. Please use RNase-free pipette tip, EP tube, etc. for the preparation of the reaction solution, and try to avoid contamination.

Experiment Process

1. Prepare the lyophilization reaction system

Amplification reaction volume	20 µl		
Lyophilization volume	12 µl*	20 µl*	
5 × One Step Mix	4 µl	4 µl	■
One Step Lyo-Enzyme Mix	1 µl	1 µl	■
3 × Excipient	4 µl*	6.7 µl*	■
Primer Forward (10 µM)	0.4 µl	0.4 µl	
Primer Reverse (10 µM)	0.4 µl	0.4 µl	
TaqMan Probe (10 µM)	0.2 µl	0.2 µl	
RNase-free ddH_2O	to 12 µl	to 20 µl	☐

The volume of each component in the reaction system can be adjusted according to the following principles:

* The lyophilization volume of each component proportionally as needed to make sure that the final concentration of the lyophilized voume is 1 ×.

▲ Generally, the final concentration of primer in the reaction system is 0.2 µM to obtain better amplification effect. When the reaction performance is poor, the primer concentration can be adjusted in the range of 0.1 - 1.0 µM.

▲ The final concentration of TaqMan Probe can be adjusted between 50 - 250 nM.

▲ The size of the amplification product should be within the range of 80 - 200 bp.

2. Lyophilization program

Steps	Temperature (°C)	Duration (min)	Vacuum degree (Pa)
Pre-chill	4		/
Freeze	4	30	/
	-40	120	/
Primary dry	-30	600	10
Secondary dry	25	360	10
	40	360	0 (Extreme Vacuum)

▲ This program is applicable to lyophilized volumes of 12 - 20 µl in the 8-tube PCR strips. If the lyophilization into lyophilized pellet or larger volume forms is required, the lyophilization procedure needs to be adjusted.

▲ Different manufacturers and models of lyophilizer may have differences, and it may be necessary to optimize the lyophilization program.

3. Lyophilized product reconstitution

Add template to lyophilized product and supplement with RNase-free ddH_2O to a final volume of 20 µl. Mix components thoroughly, then centrifuge instantaneously to collect the contents and eliminate bubbles before amplification.

4. Reaction program

Standard program (can achieve the highest amplification sensitivity)

Stage 1	Reverse Transcription	Rep: 1	50°C[a]	15 min
Stage 2	Initial Denaturation	Rep: 1	95°C	30 sec
Stage 3	Cycles	Reps: 45	95°C	10 sec
			60°C	30 sec

Fast program (can meet most applications)[b]

Stage 1	Reverse Transcription	Rep: 1	50°C[a]	5 min
Stage 2	Initial Denaturation	Rep: 1	95°C	30 sec
Stage 3	Cycles	Reps: 40	95°C	5 sec
			60°C	15 sec

a. For templates with complex secondary structures or high GC regions, increasing the reverse transcription temperature to 55°C can help improve amplification efficiency and sensitivity.

b. In the fast program, the reaction time and temperature ramp speed at each stage can be adjusted according to the Real Time PCR machine used and one's own requirements.

FAQ & Troubleshooting

FAQ	Reasons	Solutions
The lyophilized product is collapsed. 	Did not use the lyophilization program we recommend.	Operate with the supplied lyophilization excipients and in accordance with the complete lyophilization solutions we provide.
	The product reached critical temperature before the primary drying step was completed.	Decrease the temperature during the primary drying step.
	The material was not frozen completely.	Increase the time of the freezing step.
The lyophilized product is not unified and is spread out. 	Uneven temperature in the lyophilizer; sample was not in sufficient contact with the plate layer of the lyophilizer.	Performance verification of the lyophilizer; ensure sample is in full contact with the plate; decrease the freezing temperature and increase the freezing time.
	The sample was pipetted incorrectly.	Pipette the sample to the bottom of each reaction well without touching the sides of the wells or centrifuge instantaneously.
The lyophilized product is visibly shrunken. 	Insufficient primary drying, resulting in residual moisture at the bottom of the sample.	Increase the time of the primary drying or decrease the vacuum level appropriately to ensure that the sublimation efficiency of the lyophilization equipment is ≥1 mm/h; reduce the sample volume moderately.
Poor long-term storage stability of lyophilized products (moisture is present in the lyophilized product).	The lyophilized product absorbed environmental moisture because the lyophilized product was exposed for too long or the environmental humidity is too high.	Backfill the lyo chamber with dry nitrogen gas before releasing the vacuum; cap the tube before exiting the lyo chamber; store the product inside the lyophilizer until the ambient humidity is at an acceptable level.
	Lyophilized product was not sealed properly.	Ensure that the strip-tubes have been properly sealed; add desiccant, seal and store away from light.