Version: 10/2023

Lyophilization procedure

stage	steps	temperature	Slope time	Temperature control time	Vacuum degree Pa	remark
Pre-cooling	1	0 °C	10 min	30 min	--	fastest cooling rate
Pre-freeze	2	-45 °C	90 min	180 min	--	The holding time can be adjusted according to the packaging material
Sublimation drying	3	-30 °C	90 min	180 min	14	The slope time can be set to control the temperature rise
	4	-10 °C	120 min	120 min	14	
	5	0 °C	60 min	90 min	14	
Analytical drying	6	30 °C	150 min	240 min	14	

1. If the formula of auxiliary materials changes slightly, the lyophilization parameters need to be redetermined and adjusted accordingly.

2. Lyophilization equipment requirements:

The surface temperature of the cold trap coil ≤-50 °C

Layer temperature≤ -45 °C, temperature uniformity±1 °C (consult Cowin's technicians for detailed performance details and verification schemes)

Can do pressure rise test (leakage rate test before freeze-dried production)

3. Environmental requirements: solution dispensing and configuration are carried out under the protection of 10,000-level laminar flow as much as possible, because the dust in the environmental may fall into the solution to become the crystal nucleus of the lyophilization process, which affects the supercooling degree of the crystallization of the solution and leads to inconsistent product quality.

4. The temperature and humidity of the warehouse should be controlled. It is recommended that the temperature of the warehouse should be 15~25 °C, and the humidity is recommended to be ≤ 30%.

WinScript Lyo AD One Step RT-qPCR U⁺ Kit

Cat. No. : CW3354S (1 mL).
CW3354M (5 mL)

Storage Condition: -30~-15 °C storage, avoid repeated freeze-thawing.

Components

Component	CW3354S 1 mL	CW3354M 5 mL
5×WinScript Lyo AD One Step RT-qPCR U⁺ Mix	1 mL	5 mL
5×One Step lyophilized protective agent	1 mL	5 mL

Introduction

This product is a probe-based (TaqMan, Molecular Beacon, etc.) one-step Real-Time RT-qPCR kit using RNA as template. When using this product for Real Time RT-qPCR reaction, reverse transcription and quantitative PCR are performed in the same reaction system. There is no need to add reagents or open the tube lid during the reaction, avoiding contamination and improving experimental efficiency. The dUTP/UNG anti-contamination system is introduced in this reagent, which can rapidly degrade U-containing contaminants at room temperature and avoid affecting the efficiency and sensitivity of RT-qPCR. This product contains lyophilized protective agent and can be used for the preparation of lyophilized reagents.

Notes

1. This product uses RNA as a template for one-step RT-PCR experiments, so RNase contamination should be avoided during operation. It is recommended to perform RNA operations in a special area and use special instruments and consumables. Operators should wear masks and disposable gloves and change gloves frequently. Experimental related consumables should be treated with 0.1% DEPC (diethyl pyrocarbonate) aqueous solution at 37 °C for 12 hours, and autoclaved for 30 minutes before use.
2. This product should avoid repeated freeze-thawing. It is recommended to store it in separate packages.
3. If there is any precipitation or crystallization after the 5×One Step lyophilized protective agent melting, please dissolve it in a 70°C water bath. This will not affect usage effect.

Protocol

The following protocol is an example of conventional reaction systems and reaction conditions, which should be improved and optimized according to the different templates, primer structures and the size of target fragment sizes in practice.

1. Thaw the RNA template, primer probe, 5× WinScript Lyo AD One Step RT-qPCR U$^+$ Mix and 5×One Step lyophilized protectant, and place on ice for later use

2. PCR reaction system:

reagent	25 μL reaction system	Final concentration
5×WinScript Lyo AD One Step RT-qPCR U$^+$ Mix	5 μL	1×
Primer/Probe mix[1)]	X μL	
5×One Step lyophilized protective agent	5 μL	1×
RNA Template[2)]	5 μL	
Total	25 μL	

Note: 1) Generally, a better result can be obtained with a primer concentration of 0.2 μM. 0.1-1.0 μM can be set as a reference. The concentration of the probe used is related to the type of real-time PCR instruments, probe types, and fluorescent label substances used. Please refer to the instrument manual or the specific use requirements of each fluorescent probe for concentration adjustment.

2) Generally, the amount of RNA template is 10 pg-100 ng as reference. Because template contains different copies of the target gene in different species, the template can be gradient diluted to determine the optimal dosage.

3. Mix thoroughly with a vortex, centrifuge briefly, and collect the solution to the bottom of the tube.
4. RT-PCR reaction conditions:

steps	Temperature °C	Time	The number of cycles
Reverse transcriptase	50	5 min	1
Pre-denaturation	95	30 s	1
Denaturation	95	5 s	45
Annealing/extension and collecting the fluorescence	58	30 s	

Note: The annealing extension temperature can be adjusted according to the primer probe.