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5×Lyo Script One Step RT-qPCR Mix(UNG)

Product Number:PCK54

Shipping and Storage

-30°C~ -15°C.Avoid repeated freeze-thaw cycles.

Component

Component	PCK54 1mL	PCK54 5mL
5×Lyo Script One Step RT-qPCR Mix(UNG)	1mL	5mL
5×One Step lyophilization protector	1mL	5mL

Description

This product is a kit for one-step Real Time RT qPCR using RNA as a template using probe methods such as TaqMan and Molecular Beacon. When using this product for Real Time RT qPCR reaction, reverse transcription and quantitative PCR are carried out in the same reaction system, without the need to add reagents or open the tube cap, which avoids contamination and improves experimental efficiency. This reagent introduces a dUTP/UG anti contamination system, which can rapidly degrade pollutants containing U at room temperature without affecting the efficiency and sensitivity of RT qPCR. This product is equipped with a freeze-drying protectant and can be used for the preparation of freeze-drying reagents.

Note

1. This product uses RNA as a template for one-step RT-PCR experiments. During the operation, RNase contamination should be avoided. It is recommended to perform RNA operations in a dedicated area, using specialized instruments and consumables. Operators should wear masks and disposable gloves, and frequently change gloves. The experimental consumables should be treated with a 0.1% DEPC (diethyl carbonate) aqueous solution at 37 ℃ for 12 hours, and sterilized under high pressure for 30 minutes before use.
2. This product should avoid repeated freeze-thaw cycles and is recommended to be packaged and stored separately.
3. If there is precipitation or crystallization after melting the 5 × One Step Lyophilization Protector, please dissolve it in a 70 ℃ water bath without affecting its use.

Protocol

The following are examples of conventional reaction systems and conditions. In practical operation, corresponding improvements and optimizations should be made based on the differences in template, primer structure, and target fragment size.

1. Melt the RNA template、primer probe 5×Lyo Script One Step RT-qPCR Mix(UNG)、5×One Step lyophilization protector and place them on ice for later use.
2. PCR reaction system:

Reagent	25μL reaction system	Final concentration
5×Lyo Script One Step RT-qPCR Mix(UNG)	5μL	1×
Primer/Probe mix[1)]	XμL	
5×One Step lyophilization protector	5μL	1×
RNA Template[2)]	5μL	
Total	25μL	

Note:

1) Usually, a primer concentration of 0.2μM can yield good results, and a reference range of 0.1-1.0μM can be used for setting. The concentration of the probe used is related to the fluorescent quantitative PCR instrument, probe type, and

fluorescent labeling substance used. Please refer to the instrument manual or the specific usage requirements of each fluorescent probe for concentration adjustment during actual use.

2) Usually, the amount of RNA template is based on 10pg-100ng as a reference. Due to the different copy numbers of the target gene contained in templates of different species, gradient dilution of the template can be performed to determine the optimal template usage.

3. Mix well, centrifuge briefly, and collect the solution at the bottom of the tube.
4. RT-PCR reaction conditions:

Step	Temperature°C	Time	Cycles
Reverse transcription	50	5 min	1
Pre denaturation	95	30 s	1
Denaturation	95	5 s	45
Annealing extension, collecting fluorescence	58	30 s	

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

Freeze drying procedure

Stage	Step	Temperature	Slope time	Temperature control time	Vacuum degree Pa	Note
Pre cooling	1	0℃	10 min	30 min	--	The fastest at room temperature
Pre freezing	2	-45℃	90 min	180 min	--	The holding time can be adjusted according to the packaging material
Sublimation drying	3	-30℃	90 min	180 min	14	Slope time can be set to control temperature rise through slope control
	4	-10℃	120 min	120 min	14	
	5	0℃	60 min	90 min	14	
Analyze drying	6	30℃	150 min	240 min	14	

1. Minor changes have occurred in the formulation of auxiliary materials, requiring re measurement of freeze-drying parameters and corresponding adjustments.
2. Requirements for freeze-drying equipment:
   2.1. Cold trap coil surface temperature ≤ -50 ℃
   2.2. Plate layer temperature ≤ -45 ℃, temperature uniformity ± 1 ℃ (consult Kangwei technical personnel for detailed performance specifications and verification plans)
   2.3. Can be used for pressure rise testing (leak rate testing before freeze-drying production)
3. Environmental requirements: Solution packaging and preparation should be carried out under the protection of laminar flow at the 10000 level as much as possible. Dust in the environmental space can fall into the solution and become crystal nuclei during the freeze-drying process, affecting the undercooling of the solution's crystallization and resulting in inconsistent product quality.
4. The temperature and humidity of the warehouse environment should be controlled, and it is recommended that the temperature be between 15-25 ℃ and the humidity be ≤ 30%.