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Lyo Universal One Step RT-qPCR Mixture

Product Number: PCK63

Shipping and Storage

Store at 4-30°C, and store at -20°C until fully used after re dissolving. Avoid repeated freeze-thaw cycles as much as possible.

Components

Component	PCK63 48rxns	PCK63 100rxns
Lyo Universal One Step RT qPCR Mixture	6×8 well strip	1×cillin bottle

Description

Lyo Universal One Step RT qPCR Mixture is a specialized in situ all component freeze-drying reagent that uses RNA as a template for quantitative PCR reactions. The buffer contains Taq DNA polymerase modified with novel antibodies, highly efficient thermally stable reverse transcriptase, enhancers and stabilizers.Mg^{2+} and dNTP are also present, and factors that effectively inhibit non-specific PCR amplification and enhance the efficiency of multiple qPCR reactions are added. This allows for multiple fluorescence quantitative amplification reactions while ensuring primer amplification efficiency. This product is convenient and fast to use, just add primer probes, and after extraction, the nucleic acid sample can be amplified on the machine. This product is compatible with both single and multiple probe qPCR reaction systems for routine and rapid detection.

Protocol

The following examples are typical 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. Prepare an 8 well strip reaction system according to the following table, with a total volume of 25ul

Reagent	25μL reaction system	Final Conc.
Lyo Universal One Step RT qPCR Mixture	1 hole	1×
Primer probe mix[1)]	XμL	
RNA Template[2)]	XμL	
RNase-Free Water	Up to 25μL	

2. Prepare a cillin bottle reaction system according to the following table, with a total volume of 25μL.

Add 1.3ml of RNase Free Water to the cillin bottle for re dissolution, vortex mix for 10 seconds, and centrifuge briefly for later use.

Reagent	25μL reaction system	Final Conc.
Resolved Lyo Universal One Step RT qPCR Mixture	12.5μL	1×
Primer probe mix[1)]	XμL	
RNA Template[2)]	XμL	
RNase-Free Water	Up to 25μL	

Note: 1) Typically, a primer final concentration of 0.2μM can yield good results, and can be used as a reference within the set range of 0.1-1.0μM. The final concentration of the probe used is related to the fluorescent qPCR instrument used, the type of probe, and the type of fluorescent labeling substance. Please refer to the instrument manual or the specific usage requirements of each fluorescent probe for concentration adjustment during actual use.

2)Due to the different copy numbers of target genes contained in templates of different species, gradient dilution can be applied to the templates to determine the optimal template usage.

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

Conventional reaction conditions:

Step	Temperature	Time	Cycles
Reverse Transcription	50°C	5min	1
Pre denaturation	95°C	30s	1
Denaturation	95°C	10s	45
Annealing extension, collecting fluorescence	58°C[1)]	30s	

Quick response conditions

Step	Temperature	Time	Cycles
Reverse Transcription	50°C	5min	1
Pre denaturation	95°C	20s	1
Denaturation	95°C	2s	45
Annealing extension, collecting fluorescence	58°C[1)]	15s[2)]	

Note: 1) It is recommended to use a two-step PCR reaction program. If good experimental results cannot be obtained due to the use of primers with lower Tm values, a three-step PCR amplification can be attempted. The annealing temperature should be set within the range of 56°C-64°C as a reference.

2)The annealing time in the fast program can be set according to the default shortest time of the instrument used.