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Liquant™ Ultra Green qPCR Master Mix

Cat. #: M0026 Size: 5 ml/20 ml

Introduction

Liquant™ Ultra Green qPCR Master Mix, is a Taq DNA polymerase-based 2× master mix for realtime PCR, which contains all components, except for the primer. The master mix is applicable for intercalation assay with SYBR Green I and can be used in glass capillary systems or passive reference system. Hot Start technology with Taq polymerase antibodies enables high specificity and reproducible amplification. The specially optimized PCR buffer make the mix more efficient amplification of GC-rich templates and more stable at room temperature.

Package Information

Components	M0026-05 (5 ml)	M0026-20 (20 ml)
2× Liquant™ Ultra green qPCR Master Mix	5×1 ml	20×1 ml

Storage

This reagent can be stored at 2-8°C for 12 months and protected from light.

For longer storage, this reagent should be kept at -20°C and protected from light.

Primer Design

Primer length: 18~30bp

GC content of primer: 40~80%

Target length: ≤ 200 bp (optimally, ≤ 150bp)

Checking the primers:

NTC tests can distinguish unintended amplification products of primer dimers from the intended PCR products in SYBR Green I reactions. NTC test is required to verify the each of primers for assessing the extent of primer dimers.The primer of NTC with Cq<40 should be redesigned.

Prepare a dilution series with five or more dilutions of template DNA. Perform qPCR assay using the diluted DNA with the newly designed primers and draw a standard curve. Confirm that the PCR efficiency is between 95% and 105% and R2 is equal to or greater than 0.99. If the PCR efficiency or R2 are outside of these ranges, the primers concentration and reaction conditions should be optimized. If this does not improve the result, the primers should be redesigned.

Application

This reagent can be used in general detection devices, not needing ROX such as: LineGene (bioer); LightCycler (Roche); iCycler iQ, CFX96 (Biorad/MJ); Thermal Cycler Dice (Takara);

This reagent with 1× ROX can also be used in detection equipment using passive reference, such as: ABI PRISM 7000, 7700, 7900 ,7300; Step One, Step one plus *etc*. (ABI) , ABI PRISM 7500, 7500Fast (ABI); Mx3000P, 3005P, MX4000, *etc*.(Agilent).

Template DNA

Genomic DNA: Purified DNA, which would be used for general PCR, is also suitable for real-time PCR. In the case of mammalian genomic DNA, 1~10 ng genomic DNA is sufficient for real-time PCR.

cDNA: Reverse transcription reactions from total or poly (A)+ RNA may be used directly, or after dilution for realtime PCR. Before the reverse transcription reaction, it is essential to assess the extent of genomic DNA contamination with no-reverse transcription control.If genomic DNA contamination affects the Cq values, it is essential to be eliminated by DNase treatment.

Protocol

1. Preparation of the reaction mix

This premix should be fully thawed at room temperature in the bags, gently vortexed and briefly centrifuged.

Notes: Due to the high concentration stabilizer, there may be crystal precipitation in the premix, which can be used normally after being fully thawed at room temperature Purified DNA or RT reactions can be may be used directly or after dilution.

In order to reducing the artificial error of sampling, design the plate layout and sampling method by the number of the templates and primer pairs. According to the following two situations, the total reaction is divided into two parts for premixing and loading in the a thin-walled qPCR tube or plate at room temperature.

Fore more genes and less samples in one plate

Components	20μL reaction ×n	Operation
Liquant™ Ultra Green qPCR Master Mix	10μL ×n	Premix and Loading
Template DNA Dilutions	2μL ×n	
2μM Forward primer	4μL ×n	
2μM Reverse primer	4μL ×n	

Fore more samples and less genes in one plate

Components	20μL reaction ×n		Operation
Liquant™ Ultra Green qPCR Master Mix	10μL ×n		Premix and Loading
8μM Reverse primer	1μL ×n	Premix	
8μM Reverse primer	1μL ×n		
Template DNA Dilutions	8μL ×n		

Gently mix the reaction solutions and spin down in microcentrifuge.

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2. Set up the cycling conditions

2-step PCR protocol

1	Pre-denaturation	95°C	3 min	1 cycle
2	Denaturation	95°C	10 sec	40 cycles
	Template DNA Dilutions	60°C	30 sec	

Data collection should be performed at the extension step.

3-step PCR protocol

1	Pre-denaturation	95°C	5 min	1 cycle
2	Denaturation	95°C	15 sec	40c ycles
	Annealing	Tm-5°C	30 sec	
	Extension	72°C	60 sec	
3	Final Extension	72°C	5 min	1 cycle

Data collection should be performed at the extension step.

Notes:
Use this protocol first and optimize PCR conditions when necessary. Perform 3-step PCR when using primers with low Tm values or when 2-step PCR is not feasible.

The annealing temperature can be set to 55~65°C, depending on the primer Tm value.

The annealing time should be set for 5~20 seconds. Longer annealing time results increased efficiency, and a shorter time decreases non-specific amplification.

Data collection step should be longer than 10 sec.

Reference

Bustin SA, Benes V, Garson JA, *etc*,al. The MIQE guidelines: minimum information for publication of quantitative real-time PCR experiments.ClinChem. 2009, Apr; 55(4):611-22.