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Probe qPCR Master Mix

【Catalogue Number】 QPT-100

【Packing Information】 500 reactions for a total 20μL reaction volume.

【Description】

Probe qPCR Master Mix is a Taq DNA polymerase-based 2× master mix for realtime PCR, which contains all components, except for the primer and probe. This reagent is applicable in TaqMan assays or hybridization probe assays, in combination with each probe. This reagent can be used in glass capillary systems or passive reference system. Hot Start technology with anti-Taq DNA 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.

【Feature】

-High specificity: High efficiency Taq antibodies and optimized PCR buffer greatly reduce the formation of primer dimers,so it easily meets MIQE requirements

-Room-temperature stable: the performance is not easily decrease during storing and shipping.

-Wide dynamic range: the master mix demonstrates excellent reproducibility over a wide dynamic range and provides efficient amplification over 8 logs of sample input.

【Components】

QPT-100 can be used for 500 reactions for a total 20μL reaction volume.

Cat NO.	Components	Size
QPT-100	Probe qPCR Master Mix	1 mL ×5tubes/ bag

【Primer/Probe Design】

-Design of primers

Primer length: 18–25bp； Tm of primer: 60–65°C； GC content: 40–80%； Target length: 70–200 bp;

Larger targets (>200 bp) tend to reduce the efficiency and specificity of amplification.

Purification grade: OPC or HPLC grade ；

-Design of probes

Probe length: 20–30bp； Tm of probe: 65–70°C； GC content: 40–60%；

Purification grade: HPLC.

-Checking the performance of primers and probes：

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 probe, and draw a standard curve. Confirm that the PCR efficiency is between 90% and 110% and R^2 is equal to or greater than 0.99. If the PCR efficiency or R^2 are outside of these ranges, the reaction conditions should be optimized. If this does not improve the result, the primers and/or probes should be redesigned.

【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.

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【Detection】

-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).

【Protocol】

1. Preparation of the reagent

-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*

-Prepare the following reaction mixture in a 1.5mL DNase/RNase-Free centrifuge tube.

Components	μL ×n reactions		Operation
Probe qPCR Master Mix	10μL×n		Premix and Loading
10μM Reverse primer	0.8μL×n	Premix	
10μM Reverse primer	0.8μL×n		
10μM TaqMan probe	0.2μL×n		
PCR grade water	3.2μL×n		
Total Volume		15μL×n	

Notes: *The primer concentration should be optimized between 0.2-0.8μM and TaqMan® probe optimized between 0.1-0.4 μM with 10-50 copies templates per reaction . So the best primers-probe concentration sets was selected by orthogonal design of experiments.*

-Gently mix the reaction solutions and spin down in microcentrifuge.

-Loading 15μL/well in a thin-walled qPCR tube or plate.

2. Add Template DNA：

-Purified DNA or RT reactions can be may be used directly or after dilution.

-Add 5μL template DNA into the PCR tube. Please insert the tip into the reaction mix and slowly inject the template DNA.

Notes:

- If negative and positive controls are required, please add these in the order of negative control, template DNA, and positive control.

-The method of adding template DNA below the liquid level reduces template volatilization and improves the detection sensitivity of low copies templates

-Gently mix the reaction solutions and spin down in microcentrifuge.

3. Set up the qPCR cycler:

-Select the corresponding fluorescence detection channel based on the fluorescence labeling of the probe.

-The recommended 2-step PCR protocol is described below：

2-step PCR protocol				
1	Pre-denaturation	95℃	3min	1cycle
2	Denaturation	95°C	10 sec	40 cycles
	Annealing/ Extension	60°C	30 sec	
Data collection should be performed at the extension step.				

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-Perform 3-step PCR when using primers with low Tm values or when 2-step PCR is not feasible.

3-step PCR protocol				
1	Pre-denaturation	95℃	5min	1cycle
2	Denaturation	95°C	15 sec	40 cycles
	Annealing	Tm-5°C	30 sec	
	Extension	72°C	60 sec	
3	Final Extension	72°C	5min	1cycle
Data collection should be performed at the extension step.				

Notes:

-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.

【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.

【References】

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.

【Contact】

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