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TOROGreen® 5G qPCR Premix 2.0

【Catalogue Number】 QST-200

【Packing Information】 1000 reactions for a total 20μL reaction volume.

【Description】

TOROGreen®5G qPCR Premix 2.0 is an 2×master mix for intercalator-based realtime PCR with SYBRGreen I, which contains all components except for the primer. TOROIVD®5G DNA polymerase, a mutant hot-start Tth DNA polymerase modified by antibodies, allows high specificity and sensitivity for high-speed PCR reactions. The improved polymerase and reaction mixture combination also enables a high resistance to PCR inhibitors. ROX is added into the premix and can be applied to the qPCR cyclers that require a passive reference dye. The premix is suitable for high-speed PCR and enables accurate detection and quantification of targets, making it possible to obtain highly reproducible and reliable realtime PCR results over a wide dynamic range.

【Feature】

-Rapid and highly sensitive

This premix can achieve the rapid and highly sensitive quantification of a low-copy targets with probes and be suitable for the quantification of DNA viruses or cDNA at a low level.

-Room-temperature stable:

The specially optimized PCR buffer make the mix very stable at room temperature.Therefore, the performance is not easily decrease during storing and shipping.

-Inhibitor tolerant

The unique proprietary formulation of this kit allows robust performance even in the presence of substances that can normally inhibit PCR, such as heparin, hematin, or EDTA.

-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】

QST-200 can be used for 1000 reactions for a total 20μL reaction volume.

Cat NO.	Components	Size
QST-200	TOROGreen® 5G qPCR Premix 2.0	1.25 mL ×8 tubes/ Kit

【Primer Design】

- **Primer length**: 18~30bp - **GC content of primer**: 40~60% -**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 R^2 is equal to or greater than 0.99. If the PCR efficiency or R^2 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.

【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.

【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 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
TOROGreen®5G qPCR Premix 2.0	10μL×n	Premix and Loading
Template DNA Dilutions	2μL×n	
2μM Forward primer	4μL×n	Premix and Loading
2μM Reverse primer	4μL×n	

Fore more samples and less genes in one plate

Components	20μL reaction ×n		Operation
TOROGreen®5G qPCR Premix 2.0	10μL×n		Premix and Loading
8μM Reverse primer	1μL×n	Premix	
8μM Reverse primer	1μL×n		
Template DNA Dilutions	8μL×n		Premix and Loading

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

2. Set up the cycling conditions

For Bio-Rad CFX96,etc.				
1	Pre-denaturation	95℃	1min	1cycle
2	Denaturation	95°C	3sec	40 cycles
	Annealing/ Extension	60°C	5 sec	
Data collection should be performed at the extension step.				

For ABI StepOne Plus,etc.				
1	Pre-denaturation	95℃	1min	1cycle
2	Denaturation	95°C	3sec	40 cycles
	Annealing/ Extension	60°C	10sec	
Data collection should be performed at the extension step.				

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For Roche LightCycler 96 / 480 systems,etc.				
1	Pre-denaturation	95℃	1min	1cycle
2	Denaturation	95°C	10sec	40 cycles
	Annealing/ Extension	60°C	20sec	
Data collection should be performed at the extension step.				

For ABI 7500/7300 etc.				
1	Pre-denaturation	95℃	1min	1cycle
2	Denaturation	95°C	10sec	40 cycles
	Annealing/ Extension	60°C	30sec	
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 24 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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