TOROIVD TECHNOLOGY COMPANY LIMITED.
Factory: Building #20, 888 Zhujiang Road, Rudong, China.226400
Head Office: Building #C1, 880 JiangYangNan Road, Shanghai,China. 200439
Tel/ +86-21-68030217 FAX/ +86-21-33782046 Toll Free: 400-688-2055

TOROGreen® qPCR Master Mix

【Catalogue Number】 QST-100

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

【Description】

TOROGreen® 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.

According to the MIQE guidelines, a master mix should not have primer dimers amplification in NTC tests. Compared with other brands, this master mix greatly reduces the formation of primer dimers.Therefore, this premix makes qPCR primer design easier while meeting MIQE requirements.

【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】

QST-100 can be used for 1000 reactions for a total 20μL reaction volume.

Cat NO.	Components	Size
QST-100	TOROGreen® qPCR Master Mix	1 mL ×10tubes/ Kit

【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 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.

TOROIVD TECHNOLOGY COMPANY LIMITED.
Factory: Building #20, 888 Zhujiang Road, Rudong, China.226400
Head Office: Building #C1, 880 JiangYangNan Road, Shanghai,China. 200439
Tel/ +86-21-68030217 FAX/ +86-21-33782046 Toll Free: 400-688-2055

【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® qPCR Master Mix	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® qPCR Master Mix	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

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.				

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.				

TOROIVD TECHNOLOGY COMPANY LIMITED.
Factory: Building #20, 888 Zhujiang Road, Rudong, China.226400
Head Office: Building #C1, 880 JiangYangNan Road, Shanghai,China. 200439
Tel/ +86-21-68030217 FAX/ +86-21-33782046 Toll Free: 400-688-2055

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.

【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.

【Application】

[1] Liu Q , Li X , Wu R ,et al.Development of an on-spot and rapid recombinase polymerase amplification assay for Aspergillus flavus detection in grains[J].Food Control, 2021, 125(4):107957.

[2] Fei X , Ziqian W , Bingwu Y ,et al.Aldosterone alleviates lipopolysaccharide-induced acute lung injury by regulating epithelial sodium channel through PI3K/Akt/SGK1 signaling pathway[J].Molecular and Cellular Probes, 2021.

[3] Li SJ, He CF, Feng Q,,et al.Establishment of two iPSC lines from healthy donor with heterozygous mutation in the SLC26A4 gene[J].Stem Cell Research,2022,64:1873-5061.

[4] Xu K , Meng Z , Xian X M ,et al.LncRNA PVT1 induces chondrocyte apoptosis through upregulation of TNF-α in synoviocytes by sponging miR-211-3p[J].Molecular and Cellular Probes, 2020, 52:101560.

[5] Su X , Zhu Y , Hou Y ,et al.PVT1 induces NSCLC cell migration and invasion by regulating IL-6 via sponging miR-760.[J].Molecular and cellular probes, 2020, 54:101652.

[6] Xia CP , Pan T , Zhang N,et al.Sp1 promotes dental pulp stem cell osteoblastic differentiation through regulating noggin[J]. Molecular and cellular probes,2020, 50:101504.

[7] MY Xie,LH Yang,JY Cheng,et al.Gracillin relieves pulmonary fibrosis by suppressing the STAT3 axis[J]. Journal of Ethnopharmacology,2023,316:116704,

【Contact】

TOROIVD TECHNOLOGY COMPANY LIMITED.
Head Office: Building #C1, 880 JiangYang Nan Road, Baoshan,Shanghai,China. 200439.
Factory: Building #20, 888 Zhujiang Road, Rudong ,Jiangshu,China. 226400
Tel: +86-21-68030217
Mail: market@toroivd.com
http: //www.toroivd.com