TOROIVD TECHNOLOGY COMPANY LIMITED.
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TOROGreen® HRM qPCR Master Mix

【Catalogue Number】 QET-100

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

【Description】

TOROGreen® HRM qPCR Master Mix is a Taq DNA polymerase based 2×master mix for use in qPCR applications and high-resolution melting (HRM) analysis, which contains the Hot Start Taq DNA Polymerase, PCR Buffer, dNTPs, Super EvaGreen®dye, ROX, Enhancer and Stabilizer. Except for using Super EvaGreen® instead of SYBR Green I, the master mix has the same composition as TOROGreen® qPCR Master Mix(QST-100). Therefore, it has all the features of QST-100. Due to the use of Super EvaGreen®, HRM analysis can be performed .Moreover,all primers will not form dimers in the NTC tests.

【Feature】

-No primer dimer: all primers will not form dimers in the NTC tests.
-HRM analysis: HRM analysis can be performed with the master mix.
-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】

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

Cat NO.	Components	Size
QET-100	TOROGreen® HRM 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:
-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, 7500 Fast(ABI); Mx3000P, 3005P, MX4000,etc.(Agilent).

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【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® HRM 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® HRM 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

For qPCR applications (2-step PCR protocol)				
1	Pre-denaturation	95°C	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.				

For qPCR applications (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:

-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.

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For HRM analysis (3-Step Cycle)				
1	Pre-denaturation	95℃	5min	1cycle
2	Denaturation	95°C	15 sec	40 cycles
	Annealing	Tm-5°C	15 sec	
	Extension	72°C	60 sec	
3	HRM analysis Cycle	95°C	60sec	1cycle
		40°C	60sec	1cycle
		65°C	1sec	1cycle
		97°C	1sec	1cycle
Data collection should be performed at the extension step.				

Notes:

-HRM analysis cycle conditions are for reference only and shall be recommended according to the instructions of the instrument manufacturer

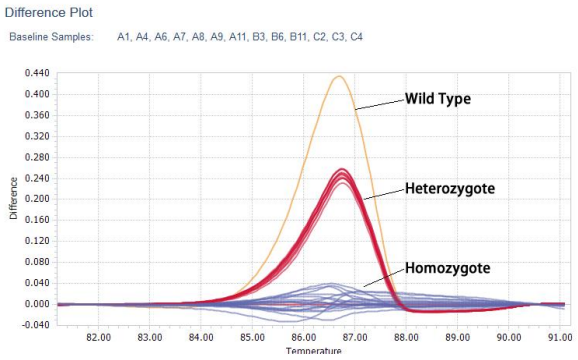
【Storage】

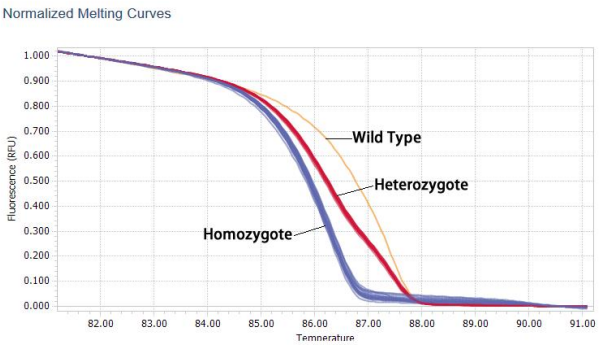
This reagent can be stored at 2-8°C for 12 months and protected from light.
For longer storage, this reagent should be kept at -20°C and protected from light.

【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】





【Contact】

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