TOROIVD TECHNOLOGY COMPANY LIMITED.
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TOROIVD®5G qPCR Premix with UNG

【Catalogue Number】 QPT-200U

【Packing Information】 **500 reactions for a total 20μL reaction volume**
(or 400 reactions for a total 25μL reaction volume).

【Description】

TOROIVD®5G qPCR Premix with UNG is a fast 2×master mix that provides for sensitive, reproducible detection up to five DNA targets. Particularly useful for virus detection with TaqMan®probe assays,this premix includes TOROIVD®5G DNA polymerase, dNTPs, dUTP,UNG and reaction buffer. The improved enzymes and reaction mixture combination also enables a high resistance to PCR inhibitors and high stability in room temperature. The premix is suitable for high-throughput analysis and can reduce the risk of cross-contamination with UNG. The premix is suitable for high-speed qPCR and enables accurate detection and quantification of targets, making it possible to obtain highly reproducible and reliable real-time 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.

-Wide dynamic range
This premix has been optimized to provide high specificity and dynamic range for use with DNA targets. This input flexibility can help streamline the number of different workflows in your lab to improve efficiency.

-Optimized for multiplexing
This premix has been validated for multiplexing up to five targets simultaneously, allowing for additional targets and/or controls to be run simultaneously for efficiency or quality control purposes.

-Avoid Contamination
This premix contains dUTP in the reaction buffer and UNG. The crossed contamination caused by PCR product can be removed so that the rate of false-positive detection can be reduced.

-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.

【Components】

QPT-200U can be used for 500 reactions for a total 20μL reaction volume or 400 reactions for a total 20μL reaction volume.

Cat NO.	Components	Size
QPT-200U	TOROIVD® 5G qPCR Premix with UNG	1.25 mL ×4 tubes/ bag

Notes: *2×5G qPCR Premix with UNG contains 0.4mM dA/C/G/T/UTP, 5mM Mg^{2+},UNG,TOROIVD®5G DNA polymerase, reaction buffer and stabilizer， etc.*

【Primer/Probe Design】

-Design of primers
Primer length: 18–25bp； Tm of primer: 60–65°C； GC content: 40–60%； 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.

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-Design for avoiding Contamination

Amplicon with high GC content will degrade the the performance of UNG for removing the contamination caused by PCR product.Therefore, when designing the primers, it is necessary to minimize the GC content of the amplicon as much as possible.

-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 R2 is equal to or greater than 0.99. If the PCR efficiency or R2 are outside of these ranges, the reaction conditions should be optimized. If this does not improve the result, the primers and/or probe should be redesigned.

【Protocol】

1. Preparation of the reagents

-Open the kit and remove the components from the box. 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.*

-One test requires 20μL or 25μL of PCR reaction mix. Depending on how many specimens will be tested, mix the required volumes of reagents as per the table below. It is advised to prepare one additional PCR reaction each time to prevent the loss of reaction mix due to pipetting error.

For 20 μL reaction volume with adding 5μL template DNA

Component	1 reaction	48 reactions	96 reactions	N reactions
TOROIVD®5G qPCR Premix with UNG	10μL	500μL	1000μL	10×(N+2)μL
Primer&Probe Premix	5 μL	250μL	500μL	5×(N+2)μL
Total	15 μL	750μL	1500μL	15×(N+2)μL

For 25 μL reaction volume with adding 10μL template DNA

Component	1 reaction	48 reactions	96 reactions	N reactions
TOROIVD®5G qPCR Premix with UNG	12.5μL	625μL	1250μL	12.5×(N+2)μL
Primer&Probe Premix	2.5 μL	125μL	250μL	2.5×(N+2)μL
Total	15 μL	750μL	1500μL	15×(N+2)μL

Notes:

-2.5mM MgCl2 final concentration have been added in this reaction mixture. But for the direct qPCR to crude template DNA,the MgCl2 concentration may need to be optimized between 2.5-5mM.

-For the direct qPCR ,adding 0.5M betaine will enhance the resistance to PCR inhibitors.

-The final concentration of primers should be optimized between 0.2μM-0.8μM and probes optimized between 0.1-0.4 μM with 10-50 copies templates per reaction. So the best primers-probe concentration sets were selected by orthogonal design of experiments.

-Mix thoroughly by vortexing and centrifuge immediately.

-Loading 15μL reaction mix per 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 for 20 μL reaction volume into the PCR tube, or 10μL template DNA for 25 μL reaction volume. Please insert the tip into the reaction mix and slowly inject the template DNA.

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

Notes:

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

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

3. Set up the qPCR cycler:

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

-Set up the universal cycling conditions for all qPCR cyclers as follows:

Universal conditions for all qPCR cyclers				
Steps		Temperature	Time	Cycles
1	UNG enzyme action	37°C	2 min	1
2	Prenaturation	95℃	3min	1
3	Denaturation	95°C	15 sec	45
	Annealing/ Extension	60°C	30 sec	
Data collection should be performed at the extension step.				

-Perform a fast cycling condition for a fast qPCR cycler as follows:

Fast condition for a fast qPCR cycler				
Steps		Temperature	Time	Cycles
1	Prenaturation	95℃	10-30sec	1
2	Denaturation	95-98°C	3-10 sec	45
	Annealing/ Extension	60-65°C	5-20 sec	
Data collection should be performed at the extension step.				

Notes:

-The indicated UNG treatment temperature can be optimized 25-37°C， and time between 0-5min.

-The indicated prenaturation temperature can be optimized 95-98°C， and time between 10sec-5min.

-The indicated denaturation temperature can be optimized 95-98°C， and time between 3sec-15sec.

-The indicated Extension /Annealing temperature can be optimized 60-65°C,and time between 5sec-30ec. Fluorescence signal gathering should be set up at this step.

【Storage】

This reagent can be stored at 2-8°C for 12 months.

For longer storage, this reagent should be kept at -20°C.

【Contact】

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