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 mix includes TOROIVD®5G DNA polymerase, dNTPs 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 mix 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.

FEATURES

-Rapid and highly sensitive

This kit 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 mRNA expressed at a low level.

-Optimized for multiplexing

This kit 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.

-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, increasing your confidence when working with a variety of complex clinical samples.

-Wide dynamic range compatible with DNA

This kit 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.

-Broad instrument compatibility

This kit can be run in either fast or standard cycling conditions with equivalent performance across a wide variety of real-time cyclers. The 50× ROX Reference dye（not supplied）is added can be applied to the real-time cyclers that require a passive reference dye.

-Avoid Contamination

This kit 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.

COMPONENTS

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

Cat NO： QPT-200U

2× 5G qPCR Premix with UNG　　1.25ml/tube，4tube /Kit

Cat NO： QPT-200U-96

2× 5G qPCR Premix with UNG　　1.25ml/tube，96tube /Kit

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.

-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 probe should be redesigned.

PROTOCOL

1. This premix should be fully thawed before use. Gently vortexed and briefly centrifuged.
2. Purified or crude template DNA can be may be used directly or after dilution.
3. Prepare the following reaction mixture in a thin- walled qPCR tube or plate.

Component	20 μL reaction	Final conc.
PCR-grade water	Up to 20 μL	N/A
2×5G qPCR Premix with UNG	10.0 μL	1×
10 μM Forward Primer	0.5μL	0.25μM
10 μM Reverse Primer	0.5μL	0.25 μM
10μM Taqman Probe	0.2μL	0.1μM
50× ROX	0/0.04/0.4μL	
Template DNA	As required	

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

Notes:

- 2.5mM $MgCl_2$ final concentration have been added in this reaction mixture. But for the direct qPCR to crude template DNA,the $MgCl_2$ concentration may need to be optimized between 2.5-8 mM.

-The primer concentration should be optimized between 0.2μM-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.

-The 50×ROX is not supplied with this kit. It is recommended that you use 50×ROX (ROX-050) optionally. 50× ROX reference dyes are used for analyses with instruments that correct for cross-talk between wells, such as the real- time PCR instruments by Applied Biosystems and Agilent Technologies. 0.4ul 50×ROX Reference Dye was added for a total 20ul reaction volume in when using the following instruments, Applied Biosystems 7300/ 7700/7900HT, StepOnePlus, etc. And 0.04ul was added for using the following instruments,Applied Biosystems 7500/7500Fast StepOne Plus , Agilent Technologies AriaMx,etc. No ROX Reference Dye is required when using other brand instruments,such as LightCycler 96 /LightCycler 480 system (Roche), CFX96 Real-Time PCR Detection System (Bio-Rad), Smart Cycler System (Cepheid) ,etc.

CYCLING CONDITIONS

The recommended 2-step PCR protocol is described below:

For Bio-Rad CFX96,etc.				
Steps		Temperature	Time	Cycles
1	UDG enzyme action	37°C	2 min	1
2	Prenaturation	95℃	1min	1
3	Denaturation	98°C	3 sec	40-45
	Annealing/ Extension	60°C	5 sec	

For Bio-Gener Q1600, etc.				
Steps		Temperature	Time	Cycles
1	UDG enzyme action	37℃	2 min	1
2	Prenaturation	95℃	1min	1
3	Denaturation	98℃	3 sec	40-45
	Annealing/ Extension	60℃	8 sec	

For ABI StepOne Plus,etc.				
Steps		Temperature	Time	Cycles
1	UDG enzyme action	37℃	2 min	1
2	Prenaturation	95℃	1min	1
3	Denaturation	95℃	3 sec	40-45
	Annealing/ Extension	60℃	10 sec	

For ABI 7500/7300 etc.				
Steps		Temperature	Time	Cycles
1	UDG enzyme action	37℃	2 min	1
2	Prenaturation	95℃	1min	1
3	Denaturation	95℃	3 sec	40-45
	Annealing/ Extension	60℃	30 sec	

For Bioer LineGene 9600 Plus, Roche LightCycler 96 /LightCycler 480 systems,etc.				
Steps		Temperature	Time	Cycles
1	UDG enzyme action	37℃	2 min	1
2	Prenaturation	95℃	1min	1
3	Denaturation	95℃	10 sec	40-45
	Annealing/ Extension	60℃	20 sec	

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

-The indicated Prenaturation temperature can be optimized 95-98℃, and time between 10sec-5min.

-The indicated denaturation temperature can be optimized 95-98℃, and time between 3sec-10sec.

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

APPLICATION DATA

Template DNA： *VP72 Gene of ASFV*

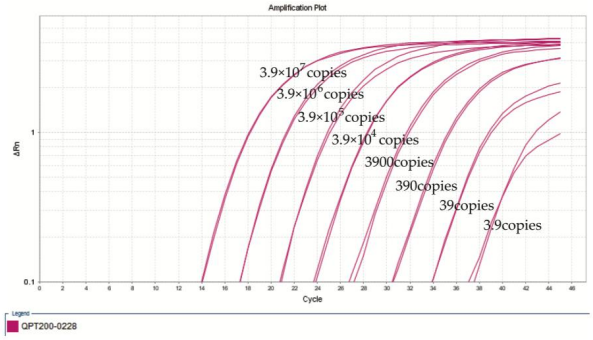
ASF-OIE-rPCR-F: *5'-CTGCTCATGGTATCAATCTTATCGA-3'*

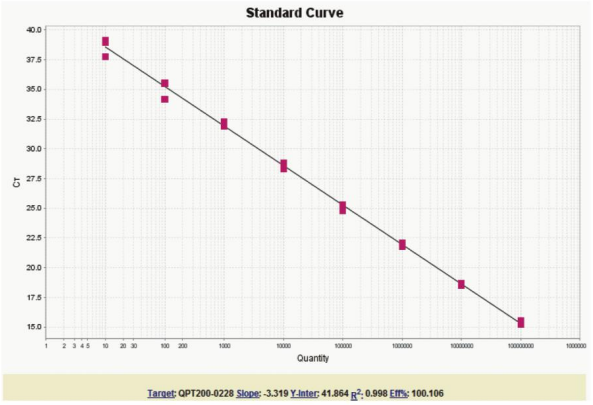
ASF-OIE-rPCR-R: *5'-GATACCACAAGATCRGCCGT-3'*

ASF-OIE-Probe: *FAM- CCACGGGAGGAATACCAACCCAGTG- TAMRA*

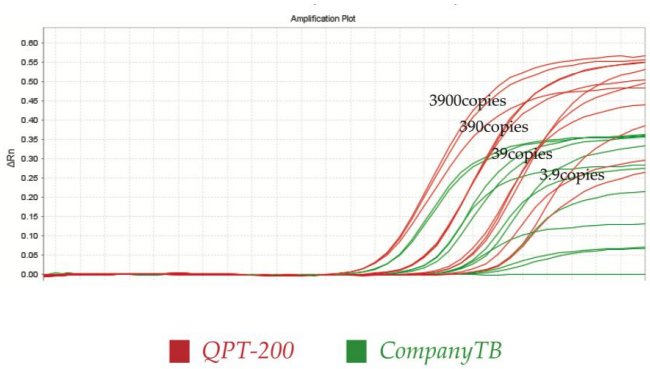
1. Wide dynamic range and high PCR efficiency.

Instrument: *StepOne Plus from Thermofisher*





2. **Sensitivity comparison with Comapany TB.**
   Instrument: *ABI 7500 from Thermofisher*



STORAGE

This reagent can be stored at 4°C for 2 months. For longer storage， this reagent should be kept at -20°C for 2 years。