RealMOD™ Probe M² 2X qPCR mix

RUO Research Use Only | REF 25359.100 Σ 100 | REF 25359.500 Σ 500 | REF 25359.1000 Σ 1000 | -25℃ -15℃

Product Description

Real-time PCR (qPCR) is the preferred method for DNA and cDNA quantification because of its high sensitivity, reproducibility and wide dynamic range. Real-time detection of PCR products makes it possible to include the reaction of fluorescent molecule that reports an increase in the amount of DNA with a proportional increase in fluorescent signal. The fluorescent chemistries employed for this purpose include DNA-binding dyes and fluorescently labeled sequence-specific primers or probes.

RealMOD™ Probe M² 2X qPCR mix is a 2X concentration premix type reagent specially designed for real time PCR by using TaqMan probe. And this kit contains all necessary reagents (Hot-StatTaq DNA Polymerase, ultrapure dNTPs, $MgCl_2$ etc.) for Real-time PCR reaction except for primers, probe and template DNA. This product has the effect of suppressing primer-dimer formation, which is especially important matter in TaqMan probe assay and this effect makes possible the accurate quantitative analysis in a wide range concentration of template DNA by minimizing non-specific amplification. RealMOD™ Probe M² 2X qPCR mix is an optimized ready-to-use solution for real time quantitative PCR assays, and the solution is activated by 10 min, incubation at 95℃. Hot-start mechanism prevents extension of non-specifically annealed primers and primer-dimer formation at low temperatures during qPCR setup.

Application

- Real-Time PCR
- Gene-expression analysis
- 3'and 5' RACE, PCR
- Pathogene detection
- cDNA library construction

Kit Contents

Product	Cat. No.	Volume	Test
RealMOD™ Probe M² 2X qPCR mix	25359.100	1 ml	100 T
	25359.500	5 ml	500 T
	25359.1000	10 ml	1,000 T

Storage And Stability

- Storage condition : Store the product at -25 ~ -15℃
- Expiration date : The solution is stable for 1 year from the date of shipping when stored and handled properly.

Instrument

- Real-time PCR Instrument
- Pipettes and Disposable Filter Tips
- Disposable Latex Gloves
- Virus DNA/RNA Extraction kit
- Desktop PCR Tube Centrifuges
- Vortex mixer

Wide Instrument Compatibility

RealMOD™ Probe M² 2X qPCR mix is designed for use with standard cycling mode on standard qPCR platforms. Our product is compatible with:

- Applied BioSystems : Quant Studio™ 12K Flex, ViiA™ 7, 7900HT, 7500, 7700, StepOne™ & StepOnePlus™
- Stratagene : MX3000P™, MX3005™
- Bio-Rad : CFX96™ & CFX384™, iQ™5 & MyiQ™, Chromo4™, Opticon® 2 & MiniOpticon®
- Qiagen : Rotor-Gene® Q, Rotor-Gene® 6000
- Eppendorf : Mastercycler®: ep realplex2 & ep realplex4
- Illumina : The Eco™
- Roche : LightCycler® 480

Precautions for Use

1. This product should be used for in research use only.
2. All procedures must be carried out in a clean bench and it is recommended that the clean bench be cleaned with alcohol after use.
3. The experimenter should wear lab coat gloves and masks and always be careful.
4. The specimen contains the risk of causing infection and unknown disease, therefore it should be careful when handling it in order to prevent infection by users and indirect contacts.
5. Do not mix reagents from different lots of this product.
6. Carefully handle the reagents and samples of this product to prevent spraying when opening the container lid and to prevent the reagents and samples from sticking to your mouth by wearing a mask.
7. While handling this product and specimens, do not place instruments that may hurt the user, such as needles or knives, and avoid accidents by not using such instruments.
8. In case of disposing of suspect specimens, contaminated test materials and instruments, should inactivate them by autoclaving, and if disinfecting, should treat them for 10 to 30 minutes using 70% ethanol and 0.5% sodium hypochlorite solution.

Nucleic acid extraction

1. Use the appropriate nucleic acid extraction kit or automated nucleic acid extraction equipment to extract nucleic acids from the sample.
2. Depending on the extraction method or kit, the yield and purification purity of the extracted nucleic acid may differ, which may affect the results of real-time PCR analysis.
3. As an automated nucleic acid extraction device, Miracle-AutoXT Nucleic Acid Extraction System (Cat.No. IMC-NC15PLUS) and the corresponding AutoXT PGS DNA / RNA Kit (Cat.No. 17168-48, 17168-96) are recommended. In case of Spin-Column Type, our Patho Gene-spin DNA / RNA Extraction Kit (Cat.No. 17154) is recommended.

Protocol

This standard protocol applies to a reaction where only template, primers, probe and water need to be added to RealMOD™ Probe M² 2X qPCR mix. To increase the reaction capacity, increase the other contents proportionally. All reagents should be thawed on ice, gently mixed and briefly centrifuged before use.

1. Thaw the RealMOD™ Probe M² 2X qPCR mix, at room temperature. Mix thoroughly and then place on ice immediately after thawing.

2. Assemble reaction tubes on ice whenever possible to avoid premature, nonspecific polymerase activity.

3. The following table shows recommended component volumes:

Reagent	20 µl Reaction*	Final Concentration
RealMOD™ Probe M² 2X qPCR mix	10 µl	1X
Forward Primer (10 µM)	0.5 – 1.0 µl	250 – 500 nM
Reverse Primer (10 µM)	0.5 – 1.0 µl	250 – 500 nM
Probe	Variable	100 – 300 nM
Template DNA	Variable	Variable
DNase/RNase free Water	Up to 20 µl	-

* When the reaction capacity is changed, the amount of 2X qRT-PCR Mix can be adjusted. For example, 50 µl reaction uses 25 µl.

4. Mix the reaction mixture by pipetting or gentle vortexing followed by a brief spin in a microcentrifuge.

5. Transfer tubes on ice into a thermal cycler pre-warmed. The following table shows recommended cycling conditions:

Steps	Temp.	Time	Cycle(s)
Initial Denaturation	95℃	5 min	1
Denaturation	95℃	5-15 sec	30 – 40
Annealing*	50℃ - 65℃**	15-60 sec	

* Signal detection step
** Cycling conditions may need to be optimized, depending on different primer and template combinations.

6. Place the PCR tubes or plates in the Real-time cycler, and start the cycling program.

7. After the reaction is completed, perform analysis.

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Belief & Relief, LiliF Diagnostics MDx Kit
QUICK GUIDE
English (영문, 英語)

LiliF Diagnostics

Performance

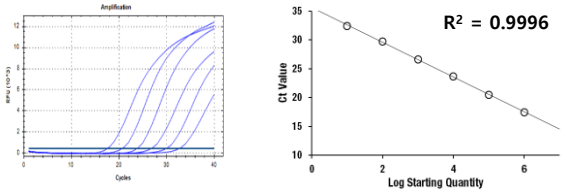

Figure 1. Amplification of ASFV plasmid DNA using RealMOD™ Probe M² 2x qPCR mix
ASFV plasmid DNA was serial diluted 1/10 (Top, Top^{-1}, Top^{-2}, Top^{-3}, Top^{-4} , Top^{-5}). Amplification of plasmid DNA using RealMOD™ Probe M² 2X qPCR mix on an CFX-96 Real-time PCR system.

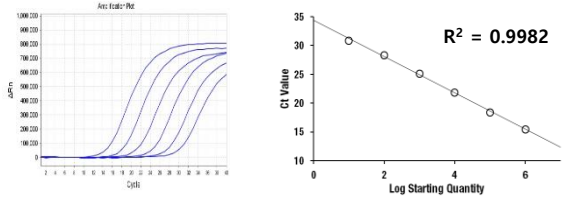

Figure 2. Amplification of salmonella plasmid DNA using RealMOD™ Probe M² 2x qPCR mix
Salmonella plasmid DNA was serial diluted 1/10 (Top, Top^{-1}, Top^{-2}, Top^{-3}, Top^{-4} , Top^{-5}). Amplification of plasmid DNA using RealMOD™ Probe M² 2X qPCR mix on an CFX-96 Real-time PCR system.

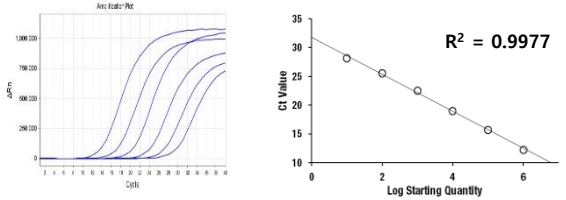

Figure 3. Amplification of listeria plasmid DNA using RealMOD™ Probe M² 2x qPCR mix
Listeria plasmid DNA was serial diluted 1/10 (Top, Top^{-1}, Top^{-2}, Top^{-3}, Top^{-4} , Top^{-5}). Amplification of plasmid DNA using RealMOD™ Probe M² 2X qPCR mix on an CFX-96 Real-time PCR system.

Trouble Shooting Guide

This troubleshooting guide may be helpful in solving problems that may frequently arise. The scientists at iNtRON are always happy to answer any questions you may have about the information or protocol in this manual or other molecular biology applications.

Problem / Possible cause	Recommendation
No Product, or weak product signal in qPCR	
1) Pipetting error or missing reagent	• Check the concentrations and storage conditions of the reagents, including primers, template DNA. Repeat the qPCR.
2) No detection activated	• Check that fluorescence detection was activated in the cycling program.
3) Problems with starting template	• Check the concentration, storage conditions, and quality of the starting template
4) Insufficient number of cycles	• Increase the number of cycles.
5) Annealing temperature too high	• Decrease annealing temperature in steps of 2℃.
6) Annealing temperature too low	• Increase annealing temperature in steps of 2℃.
7) Incorrect setting for sample position.	• Reposition the sample tubes.
8) Incorrect setting for data collection	• Confirm the data collection setting.
Variation in detection	
1) Inappropriate concentration of primers	• Optimize primer concentration according to the instructions.
2) Failure or malfunction of device	• Check the device.
3) Variation of dispensed volume	• Increase the reaction volume.
4) Inappropriate cycle conditions	• Confirm Tm of the primers.
Poor dynamic range of CT value	
1) Template amount too high	• Do not exceed maximum recommended amount of template.
2) Template amount too low	• Increase template amount, if possible.
Signals in blank reactions	
1) Contamination of amplicons or sample DNAs	• Use fresh PCR grade water. Re-make primer solution and master mix.
2) Detection of a non-specific amplification	• Optimize the primer and cycle conditions.
Primer-dimmers and/or nonspecific PCR Products	
1) To much amount of primer	• Decrease the amount of primer.

Related Products

Cat. No.	Product	Size
17168-48	AutoXT PGS DNA/RNA Kit (Individual)	48 T
17168-96	AutoXT PGS DNA/RNA Kit (Well plate)	96 T
17154	Patho Gene-spin™ DNA/RNA Extraction Kit	50 col.
17151	Viral Gene-spin™ Viral DNA/RNA Extraction Kit	50 col.

LiliF Diagnostics

EXPLANATION OF SYMBOLS

Manufactured by | Attention | Consult Instructions For Use

Manufacturing date | Expire date | Keep away from sunlight

Sufficient for tests | Do not reuse | Storage temperature limitation

LOT Batch number | RUO Research use only | REF Product number

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