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RealMOD™ Probe M² 2X qRT-PCR mix (with UDG)

RUO Research Use Only | REF 25361.100 Σ 100 | REF 25361.500 Σ 500 | REF 25361.1000 Σ 1000 | -15℃ -25℃

Product Description

Real-time RT-PCR (qRT-PCR) is the preferred method for RNA quantification because of its high sensitivity, reproducibility and wide dynamic range. Recently, the importance of accuracy has emerged in molecular diagnosis by using Real-time RT-PCR.

RealMOD™ Probe M² 2X qRT-PCR mix (with UDG) is a 2X concentration premix type reagent specially designed for Real-time RT-PCR by using TaqMan probe. And this kit contains all necessary reagents (DNA Polymerase, UDG, reverse transcriptase, ultrapure dNTPs, dUTP, $MgCl_2$ etc.) for Real-time RT-PCR reaction except for primers, probe and template RNA. The added UDG-system reagents, dUTP and thermolabile UDG, are included in the mixture to prevent the reamplification of cross/carry over PCR products between reactions. dUTP in the mixure ensures that any amplified cDNA will contain uracil. UDG removes uracil residues from single- or double-stranded cDNA, preventing uracil containing cDNA from serving as template in future PCRs. Also, the added anti Taq antibody based on Hot-start DNA polymerase prevents extension of non-specifically annealed primers and primer-dimer formation at low temperatures during qRT-PCR setup. Thus, this RealMOD™ Probe M² 2X qRT-PCR mix (with UDG) enables accurate and convenience quantitative analysis over a wide range of template RNA concentrations. A ready-to-use solution is optimized for Real-time qRT-PCR analysis.

Application

- Real-Time RT-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 qRT-PCR mix (with UDG)	25361.100	1 ml	100 T
	25361.500	5 ml	500 T
	25361.1000	10 ml	1,000 T

- 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

Precautions for Use

1. This product must 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 must wear lab coat gloves and mask and always be careful.
4. The specimen contains the risk of causing infection and unknown disease, therefore it must 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 to prevent from spraying when opening the container lid and 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, must inactivate them by autoclaving, and if disinfecting, must treat them for 10 to 30 minutes using 70% ethanol and 0.5% sodium hypochlorite solution.

Protocol

This standard protocol applies to a reaction in which only template, primers, probe and water need to be added to RealMOD™ Probe M² 2X qRT-PCR mix (with UDG). 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 qRT-PCR mix (with UDG), at room temperature. Mix thoroughly and then place on ice immediately after thawing.

2. Assemble reaction tubes on ice to avoid nonspecific polymerase activity.

3. The following table shows recommended component volumes.

Reagent	20 µl Reaction*	Final Concentration
RealMOD™ Probe M² 2X qRT-PCR mix (with UDG)	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 RNA	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)
UDG reaction	25℃	5 min	1
Reverse Transcription	50℃	10 min	1
Initial Denaturation	95℃	10 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 plate in the Real-time cycler, and start the cycling program.

7. After the reaction is completed, perform analysis.

Performance

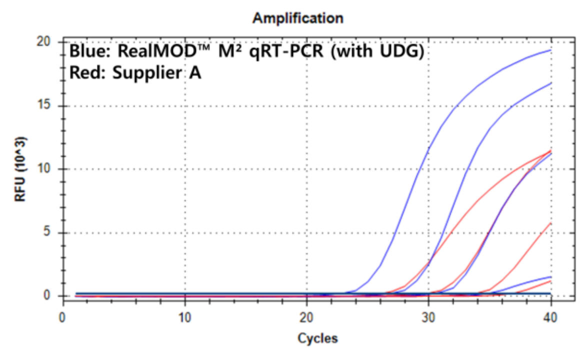

Figure 1. Performance comparison test.
Real-time RT-PCR results; RealMOD™ Probe M² 2X qRT-PCR (with UDG) has excellent Ct value and dynamic range.
- Template : 10 fold serially diluted SARS CoV-2 RNA (10^4, 10^3, 10^2, 10 copy/µl).
- Target gene : RdRP gene of SARS CoV-2.

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

LiliF Diagnostics

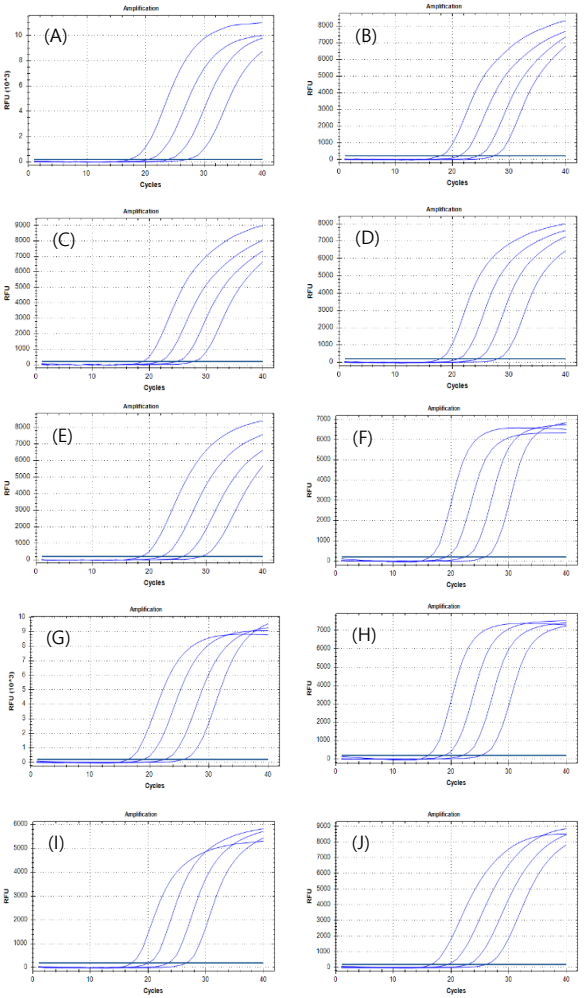

Figure 2. Amplification of various RNA using RealMOD™ Probe M² 2x qRT-PCR mix (with UDG).
Various RNA samples were diluted serially(10^6, 10^5, 10^4, 10^3 copy/μl). Amplification of samples were tested using RealMOD™ Probe M² 2X qRT-PCR mix (with UDG) on an CFX-96 Real-time PCR system.
(A: Rift Valley fever virus, B: Ebola virus, C: Marburg virus, D: Junin virus, E: Machupo virus, F: Sabia virus, G: Omsk hemorrhagic fever virus, H: Chapare virus, I: Yellow fever virus, J: Chikungunya virus)

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 qRT-PCR**	
Pipetting error or missing reagent	• Check the concentrations and storage conditions of the reagents, including primers, template RNA. Repeat the qRT-PCR.
Instrument settings are incorrect	• Check the correct instrument settings (dye selection, reference dye, number of cycles and so on).
Problems with starting template	• Confirm RNA degradation by bioanalyzer and replace RNA if necessary.
cDNA synthesis temperature too high/low priming efficiency	• RealMOD™ Probe M² 2X qRT-PCR mix (with UDG) in this formulation typically operates in a temperature range of 50°C–65°C.
Incorrect setting for sample position.	• Reposition the sample tubes.
Template amount too high/low	• Do not exceed range recommended amount of template.
❖ **Variation in detection**	
Inappropriate concentration of primers	• Optimize primer concentration according to the instructions.
Failure or malfunction of device	• Check the device.
Variation of dispensed volume	• Increase the reaction volume.
Inappropriate cycle conditions	• Confirm Tm of the primers.
❖ **Signals in no-template controls**	
Template or reagents are contaminated by nucleic acids	• Use fresh PCR grade water. Re-make primer solution and master mix.
Detection of a non-specific amplification	• Optimize the primer and cycle conditions.
Primer-dimmers and/or nonspecific PCR products	• Using validated pre-designed primer/probe sets.

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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