RealMOD™ Probe M² 2X qRT-PCR mix

RUO Research Use Only | REF 25358.100 Σ100 | REF 25358.500 Σ500 | REF 25358.1000 Σ1000 | -25℃ ~ -18℃

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. Real-time detection of PCR products makes it possible to include the reaction of fluorescent molecule that reports an increase in the amount of RNA 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 qRT-PCR mix 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 (RT enzyme, DNA Polymerase, ultrapure dNTPs, $MgCl_2$ etc.) for Real-time RT-PCR reaction except for primers, probe and template RNA. The added anti Taq antibody based Hot-start DNA polymerase is prevents extension of non-specifically annealed primers and primer-dimer formation at low temperatures during qRT-PCR setup. And The added reverse transcriptase is an improved version, M-MLV Reverse, which lacks RNaseH activity and has high thermal stability. Therefore Thus, this RealMOD™ Probe M² 2X qRT-PCR mix enables accurate quantitative analysis over a wide range of template RNA concentrations. A ready-to-use solution optimized for real-time quantitative RT-PCR analysis.

Application

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

Kit Contents

Cat. No.	Product	Volume	Test
25358.100	RealMOD™ Probe M² 2X qRT-PCR mix	1 ml	100 T
25358.500		5 ml	500 T
25358.1000		10 ml	1,000 T

Storage And Stability

- Storage condition : Store the product at -20℃
- 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 qRT-PCR mix is designed for use with standard cycling mode on standard qRT-PCR 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 qRT-PCR 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 qRT-PCR 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 qRT-PCR 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 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)
Reverse Transcription	50℃	10-30 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 plates in the Real-time cycler, and start the cycling program.

7. After the reaction is completed, perform analysis.

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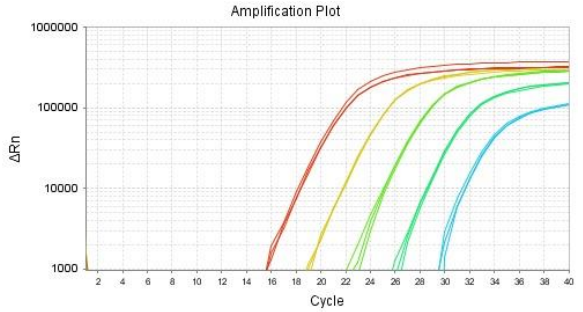
Belief & Relief, LiliF Diagnostics MDx Kit
QUICK QUIDE
English (영문 英語)

LiliF Diagnostics

Performance



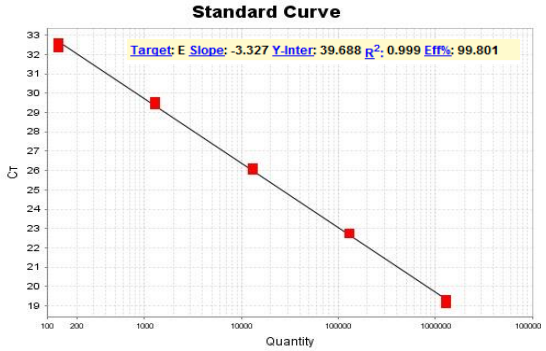


Figure 1. Amplification of SARS CoV-2 RNA using RealMOD™ Probe M² 2x qRT-PCR mix
SARS CoV-2 RNA was serial diluted 1/10, 10^6, 10^5, 10^4, 10^3, 10^2. Amplification of SARS CoV-2 RNA using RealMOD™ Probe M² 2X qRT-PCR mix on an ABI 7500 fast Real-time PCR system.
Real-time RT-PCR results; slope : -3.327, R2 : 0.999, PCR efficiency 99.8%

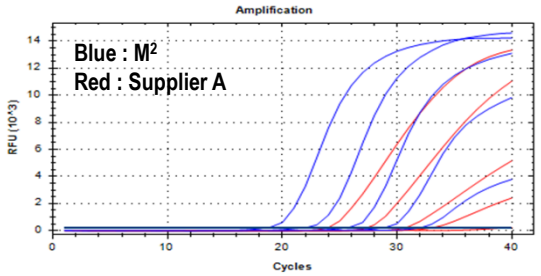


Figure 2. Performance comparison test with other products.
Real-time PCR amplification of the RdRP gene of coronavirus. SARS CoV-2 RNA was serial diluted 1/10, 10^6, 10^5, 10^4, 10^3, 10^2. Amplification of SARS CoV-2 RNA using RealMOD™ Probe M² 2X qRT-PCR mix on an Bio-Rad CFX96 Real-time PCR system.
Real-time RT-PCR results; M² 2X qRT-PCR has excellent ct value and dynamic range.

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	
1) Pipetting error or missing reagent	• Check the concentrations and storage conditions of the reagents, including primers, template RNA. Repeat the qRT-PCR.
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.
17221	easy-spin™ Total RNA Extraction Kit	50 col.
17061	easy-BLUE™ Total RNA Extraction Kit	100 ㎕

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

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