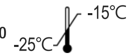
RealMOD™ Probe M^2 2X qPCR mix (with UDG)

RUO Research Use Only | REF 25360.100 Σ 100 | REF 25360.500 Σ 500 | REF 25360.1000 Σ 1000 | -25℃ – -15℃



Product Description

Real-time PCR (qPCR) is the preferred method for DNA quantification because of its high sensitivity, reproducibility and wide dynamic range. Recently, the importance of accuracy such as false positive has emerged in molecular diagnosis by using Real-time PCR.

RealMOD™ Probe M^2 2X qPCR mix (with UDG) is a 2X concentration premix type reagent specially designed for Real-time PCR by using TaqMan probe. And this kit contains all necessary reagents (DNA Polymerase, ultrapure dNTPs, dUTP, $MgCl_2$ etc.) for Real-time PCR reaction except for primers, probe and template DNA. 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 DNA will contain uracil. UDG removes uracil residues from single- or double-stranded DNA, preventing uracil containing DNA from serving as template in future PCRs. Also, 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 qPCR setup. Thus, this RealMOD™ Probe M^2 2X qPCR mix (with UDG) enables accurate and convenience quantitative analysis over a wide range of template DNA concentrations. A ready-to-use solution is optimized for Real-time quantitative PCR analysis.

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 M^2 2X qPCR mix (with UDG)	25360.100	1 ml	100 T
	25360.500	5 ml	500 T
	25360.1000	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 M^2 2X qPCR mix (with UDG) 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 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.

Nucleic acid extraction

1. Use the appropriate nucleic acid extraction kit or automated nucleic acid extraction equipment to extract nucleic acid from the sample.
2. Depending on the extraction method, 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 in which only template, primers, probe and water need to be added to RealMOD™ Probe M^2 2X qPCR 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 M^2 2X qPCR 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 M^2 2X qPCR 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 DNA	Variable	Variable
DNase/RNase free Water	Up to 20 µl	-

* When the reaction capacity is changed, the amount of 2X qPCR 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
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.

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iNtRON Biotechnology
(# 701- 704, Jung-Ang Induspia V, Sangdaewon-dong)
137, Sagimakgol-ro, Joongwon-gu, Sungnam-si, Gyeonggi-do, Korea
(주)인트론바이오테크놀로지
경기도 성남시 중원구 사기막골로 137
(상대원동, 중앙인더스피아 5차 701~704호)

Customer & Technical Service
Do not hesitate to ask us any question
shop.intronbio.com
Tel : +82-505-550-5600
Fax : +82-505-550-5660
Mail : intronbio@intronbio.com

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

Performance

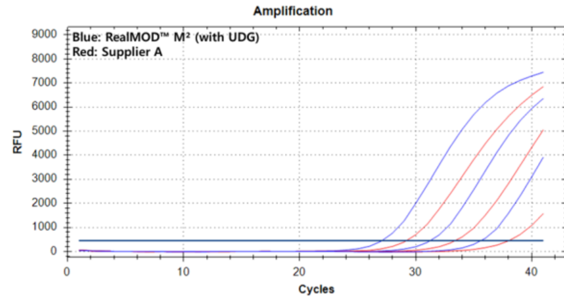

Figure 1. Performance comparison test with other product.
ASFV standard DNA was serial diluted 1/10 (1.54×10^4, 1.54×10^3, 1.54×10^2). Amplification of ASFV standard DNA using RealMOD™ Probe M² 2X qPCR mix (with UDG) on an CFX-96 Real-time PCR system.
Real-time PCR results; RealMOD™ Probe M² 2X qPCR (with UDG) has excellent Ct value and dynamic range.

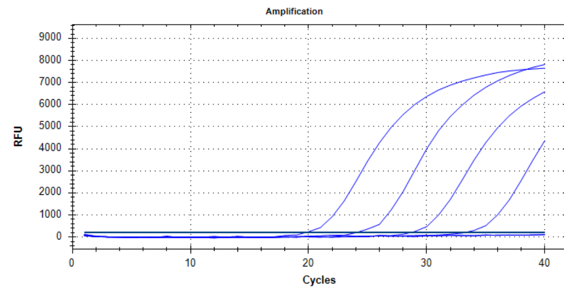

Figure 2. Amplification of salmonella plasmid DNA using RealMOD™ Probe M² 2x qPCR mix (with UDG)
Salmonella standard DNA was serial diluted 1/10 (6.18×10^5, 6.18×10^4, 6.18×10^3, 6.18×10^2). Amplification of salmonella standard DNA using RealMOD™ Probe M² 2X qPCR mix (with UDG) on an CFX-96 Real-time 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.

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EXPLANATION OF SYMBOLS

LOT Batch number
RUO Research use only
REF Product number
Sufficient for tests
Do not reuse
Storage temperature limitation
Manufacturing date
Expire date
Keep away from sunlight
Manufactured by
Attention
Consult Instructions For Use

• Document No. : QG-25360-R00
• Date : 2021. 06. 16