RealMOD™ Probe R² 2X qPCR mix (with UDG)

RUO Research Use Only | REF 25363.200 Σ 200 | REF 25363.500 Σ 500 | REF 25363.1000 Σ 1000 | -20℃

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

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 has emerged in molecular diagnosis by using Real-time PCR.

RealMOD™ Probe R² 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, UDG, 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 re-amplification of cross/carry over PCR products between reactions. dUTP in the mixture ensures that any amplified DNA will contain uracil. UDG can removes uracil residues from single- or double-stranded DNA, preventing uracil containing DNA from serving as template in future PCRs. Also, the Hot-start DNA polymerase based on antibody prevents extension of non-specifically annealed primers and primer-dimer formation at low temperatures during qPCR setup. Thus, this RealMOD™ Probe R² 2X qPCR mix (with UDG) enables accurate and convenient quantitative analysis over a wide range of template DNA concentrations. A ready-to-use solution is optimized for Real-time qPCR analysis.

Application

- Gene-expression analysis
- Pathogen detection
- Detection and quantification of DNA target

Kit Contents

Product	Cat. No.	Volume	Test
RealMOD™ Probe R² 2X qPCR mix (with UDG)	25363.200	1.1 ml x 2 vials	200 T
	25363.500	1.1 ml x 5 vials	500 T
	25363.1000	1.1 ml x 10 vials	1,000 T

Storage and Stability

- Storage condition : Store below -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

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 is cleaned with alcohol after use.
3. The experimenter must wear lab coat gloves, mask and always be careful.
4. The specimen might 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 be inactivated them by autoclaving
9. 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 needed to be added to RealMOD™ Probe R² 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 R² 2X qPCR mix (with UDG) at room temperature.

2. Mix thoroughly and then place on ice immediately after thawing.

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

4. The following table shows recommended component volumes.

Reagent	20 µl Reaction*	Final Concentration
RealMOD™ Probe R² 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, in using 50 µl, you can use 25 µl.

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

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

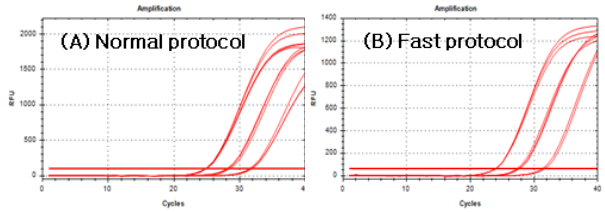
Steps	Temp.	Time	Cycle(s)
UDG reaction	25℃	5 min	1
Initial Denaturation	94℃	2-5 min	1
Denaturation	94℃	5-15 sec	30 – 40
Annealing*	50℃ - 65℃**	15-60 sec	

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

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

8. After the reaction is completed, perform analysis.

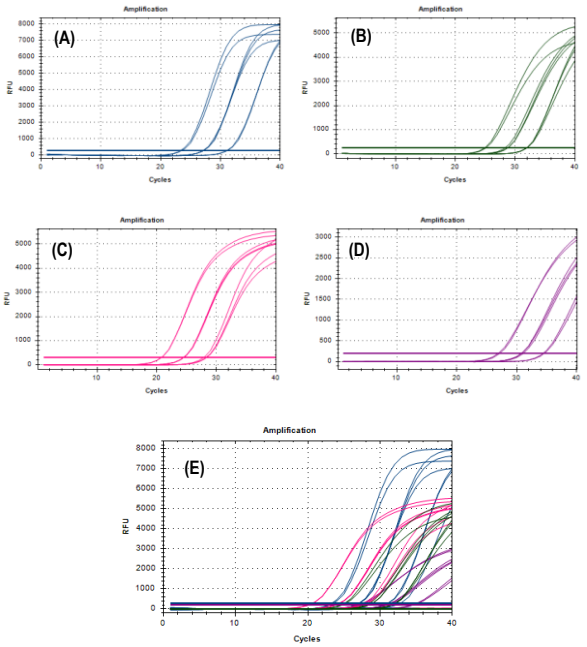
Performance



	Normal condition (96 min)			Fast condition (50 min)		
Dilution	10^{-1}	10^{-2}	10^{-3}	10^{-1}	10^{-2}	10^{-3}
Ct_{avg}	24.87	28.23	31.75	24.69	28.00	31.63
Linearity	0.9998			0.9992		
Efficiency	103.60 %			104.51 %		

Figure 1. Performance comparison between normal and fast protocols using RealMOD™ Probe R² 2x qPCR mix (with UDG).
Applying fast PCR condition using RealMOD™ Probe R² 2X qPCR (with UDG), the same detection performance was confirmed compared to normal PCR conditions.
- Template : *Treponema varginallis* DNA 10-fold dilution serially
- (A) Normal PCR condition (96 min) (B) Fast PCR condition (50 min)

LiliF Diagnostics



Target	Dilution (Ct_{avg})			Linearity	Efficiency
	10^{-1}	10^{-2}	10^{-3}		
Target 1	23.28	26.94	30.64	1.0000	110.78 %
Target 2	25.01	28.59	31.96	0.9997	104.61 %
Target 3	20.72	24.10	27.71	0.9997	105.29 %
Target 4	27.05	30.92	34.58	0.9997	113.41 %

Figure 2. Multiplex PCR of various DNA using RealMOD™ Probe R² 2x qPCR mix (with UDG).

The Multiplex PCR amplification performance of the RealMOD™ Probe R2 2X qPCR mix was evaluated using various types of viral DNA by 10-fold dilution serially (Using CFX-96 Real-time PCR system). (A: *C.trachomatis* DNA , B: *N.gonorrhoeae* DNA, C: *T.vaginalis* DNA, D: *M.genitalium* DNA, E : 4 plex Multiplex PCR)

TroubleShooting 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 the information or protocol in this manual or other molecular biology applications.

Problem / Possible cause	Recommendation
❖ **No or weak signal in qPCR**	
Pipetting error or missing reagent	• Check the concentrations and storage conditions of the reagents, including primers, template DNA. Repeat the qPCR.
Incorrect Instrument settings	• Check the correct instrument settings (dye selection, reference dye, number of cycles and so on).
Problems with starting template	• Confirm DNA degradation by bioanalyzer and replace DNA if necessary.
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 volume of the template. (ex. 1uL→5uL)
Inappropriate cycle conditions	• Confirm Tm of the primers.
❖ **Signals in no-template control**	
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.

Ordering Information

Product Name	Amount	Cat. No.
RealMOD™ Probe M² 2X qPCR mix (with UDG)	100 rxn.	25360.100
	500 rxn.	25360.500
	1,000 rxn.	25360.1000
RealMOD™ Probe M² 2X qPCR mix	100 rxn.	25359.100
	500 rxn.	25359.500
	1,000 rxn.	25359.1000
Patho Gene-spin™ DNA/RNA Extraction Kit	50 col.	17154
Miracle-AutoXT Automated Nucleic Acid Extraction System	-	IMC-NC15PLUS
AutoXT PGS DNA/RNA Kit	48 tests	17168-48
	96 tests	17168-96
AutoXT CLiNiC-Q multi DNA Kit	48 tests	17601-48
	96 tests	17601-96

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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