RealMOD™ Probe W² 2x qRT-PCR mix

For Real-time quantitative RT-PCR

RUO *Research Use Only* REF *25352* Σ 100 -22 ℃ -18 ℃

INTRODUCTION

RealMOD™ Probe W² 2x qRT-PCR mix is an optimized ready-to-use solution for one- step RT qPCR using TaqMan probe for detection. For reactions, simply add template RNA, probe and a pair of primers resuspended in water. A reverse transcriptase included in this product is a n improved version of M-MLV Reverse Transcriptase. It lacks RNaseH activity, and Is highly h eat stable. Therefore, this product is most suitable for efficiently synthesizing long cDNA by i ncubating reactions at elevated temperature(50℃) since high temperature helps removal of secondary structure present in long RNA transcripts. Compared to other suppliers worldwide, this product can synthesize cDNA with much (approximately 100 fold) less amounts of total RNA. This become more prominent as cDNA to be synthesized is long. In addition, HOT Start PCR polymerase which is greatly improved in both fidelity and yield for polymerase chain reac tion.

KIT CONTENTS

Label	Volume
RealMOD™ Probe W² 2x qRT-PCRmix	100 rxn.

† Spin down before use

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 an d handled properly.

APPLICATIONS

- Real-time RT-PCR
- Gene expression profiling
- Array validation
- Gene knockdown verification

NOTICE BEFORE USE

The RealMOD™ Probe W² 2x qRT-PCR mix is intended for research use only. This product is n ot intended for the diagnosis, prevention, or treatment of disease. All due care and attention s hould be exercised in the handling of the products. Do not use internally or externally in huma ns or animals. Please observe general laboratory precaution and utilize safety while using thi s kit.

PROTOCOL

This standard protocol applies to a single reaction where only template, primers, probe and w ater need to be added to the RealMOD™ Probe W² 2x qRT-PCR mix. For multiple reactions, sc ale-up volume of reaction components proportionally. All reagents should be thawed on ice, g ently mixed and briefly centrifuged before use.

1. **Thaw the RealMOD™ Probe W² 2x qRT-PCR mix, at room temperature. Mix thoroughly an d then place on ice immediately after thawing.**
2. **Assemble reaction tubes on ice whenever possible to avoid premature, nonspecific poly merase activity.**
3. **The following table shows recommended component volumes:**

Component	20 µl Reaction	50 µl Reaction	Final Concentration
RealMOD™ Probe W² 2x qRT-PCR mix	10 µl	25 µl	1X
Forward Primer (10 µM)	0.2 – 2.0 µl	0.5 – 5.0 µl	0.1 – 1.0 µM
Reverse Primer (10 µM)	0.2 – 2.0 µl	0.5 – 5.0 µl	0.1 – 1.0 µM
Fluorescence Probe	Variable	Variable	Variable
Template RNA	≥ 1 µl	≥ 1 µl	As needed
DNase/RNase free Water	Up to 20 µl	Up to 50 µl	-

* In general, use primers greater than 0.5 µM for sensitivity and less than 0.5 µM for specificity.

4. **Mix the reaction mixture by pipetting or gentle vortexing followed by a brief spin in a mic rocentrifuge.**
5. **Optional-Overlay reactions with one-half volume PCR-grade mineral oil when not using h eated lid on thermal cycler.**
6. **Transfer tubes on ice into a thermal cycler pre-warmed. The following table shows recom mended cycling conditions:**

Steps	Temp.	Time	Cycle(s)
Reverse Transcription	45-50℃	10-30min	1
Initial Denaturation*	95℃	10min*	1
Denaturation	95℃	20 sec	25 - 40
Annealing	50℃ - 65℃	40 sec	
Elongation	72℃	30 sec	
Final Extension	72℃	5min.	1

* Cycling conditions may need to be optimized, depending on different primer and template combinations. For example, raise the annealing temperature to prevent non-specific primer binding, increase extension time to generate longer PCR products.

USE OF THE ROX REFERENCE DYE

ROX reference dye is not included in this kit and may be added to compensate for non- PCR re lated variations in fluorescence. Addition of the reference dye is optional. Optimizing the ROX dye concentration within the qRT-PCR reaction is an important aspect of setup. Too much RO X in the qRT-PCR reaction will reduce background but also makes a low target signal difficult to distinguish from background.

WIDE INSTRUMENT COMPATIBILITY

RealMOD™ Probe W² 2x qRT-PCR mix is designed for use with standard cycling mode on stan dard 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

GENERAL CONSIDERATION

1. Primer design guidelines
   The specific amplification, yield and overall efficiency of any Real-time PCR can be criticall y affected by the sequence and concentration of the primers, as well as by the amplicon le ngth. We strongly recommend taking the following points into consideration when designi ng and running your Real-time PCR.
   1) Use primer-design software, such as Primer3 (http://frodo.wi.mit.edu/primer3/) or vis ual OMPTM (http://dnasoftware.com/).
   2) GC contents should be between 30% and 80% (ideally 40-60%).
   3) Avoid runs of identical nucleotides, especially of 3 or more Gs or Cs at the 3' end.
   4) The Tm should be between 58℃ and 60℃.
   5) Keep the GC contents in the 30-80% range.
   6) Avoid runs of identical nucleotides. If repeats are present, there must be fewer than fo ur consecutive G residues.
   7) Make sure the five nucleotides at the 3' end contain no more than two G and/or C bas es.

2. Primer design guidelines
   It is important to detect the presence of contaminating DNA that may affect the reliability of the data. Always include a no-template control (NTC), replacing the template with PCR grade water.

TROUBLE SHOOTINGGUIDE

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 infor mation 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 g reagent	• Check the concentrations and storage conditions of the reagents, missin 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 t emplate	• Check the concentration, storage conditions, and quality of the starting t emplate. If necessary, make new serial dilutions of template DNA from t he stock solutions. Repeat the PCR using the new dilutions.
	• Increase the number of cycles.
4) Insufficient number of c ycles	
5) Annealing temperature too high	• Decrease annealing temperature in steps of 2℃.
6) Annealing temperatur e 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 concentratio n of primers	• Optimize primer concentration according to the instructions.
2) Failure or malfunction of devic e	• Check the device.
3) Variation of dispensed volume	• Increase the reaction volume.
4) Inappropriate cycle nditions	• Confirm Tm of the primers. co
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 mplicons or sample DNAs	• Use fresh PCR grade water. Re-make primer solution and master a mix.
2) Detection of a non-specific amplification	• Optimize the primer and cycle conditions.
Primer-dimmers and/or nonspecific PCR Products	
1) Annealing too low	• Temperature increase annealing temperature in increments of 2℃.
	• Decrease the amount of primer.
2) To much amount of p rimer	

TERMS USED IN REAL-TIMEPCR

Term	Definition
Baseline	The initial cycles of Real-time PCR in which there is little or no change in fluorescence signal.
Threshold	A level of ΔRn - automatically determined (or manually set) by the Real-time PCR system software – used for Ct determination in real time assays. The level is set to be above the baseline and sufficiently low to be within the exponential growth region of the amplification curve. The threshold is the line whose intersection with the amplification plot defines the Ct.
Threshold cycle (Ct)	The fractional cycle number at which the fluorescence passes the threshold value.
Passive reference	A dye that provides an internal fluorescence reference to which the reporter dye signal can be normalized during data analysis. Normalization is necessary to correct for fluorescence fluctuations caused by changes in concentration or in volume. Passive reference can be optionally detected on certain real- time instruments (CCD detector type).
Normalized reporter (Rn)	The ratio of the fluorescence emission intensity of the reporter dye to the fluorescence emission intennsity of the passive reference dye.
Delta Rn (ΔRn)	The magnitude of the signal generated by the specified set of PCR conditions (ΔRn = Rn - baseline).

ORDERING INFORMATION

Product Name	Amount	Cat. No.
Fast HQ RNA Extraction Kit	50 col.	17213
easy-spin™ Total RNA Extraction Kit	50 col.	17221
IQeasy™ Plus Plant RNA Extraction Mini Kit	50 col.	17491
RNA-spin™ Total RNA Extraction Kit	50 col.	17211
easy-BLUE™ Total RNA Extraction Kit	5 x 100 ml	17061L
easy-BLUE™ Total RNA Extraction Kit	100 ml	17061
RealMOD™ Green qRT-PCR mix (LR)	100 rxn.	25107
RealMOD™ Green qRT-PCR mix	100 rxn.	25109

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