RealMOD™ Green W² 2x qPCR mix

For Real-time quantitative PCR

RUO *Research Use Only* REF 25350 Σ 100 -22℃ -18℃

INTRODUCTION

Real-time PCR (qPCR) is the preferred method for DNA and cDNA 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 DNA 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™ Green W² 2x qPCR mix is an optimized ready-to-use solution for real-time quantitative PCR assays, incorporating SYBR Green I dye. It comprises all the components necessary to perform qPCR: Taq DNA Polymerase, ultrapure dNTPs, $MgCl_2$ and SYBR Green I dye. The user simply needs to add water, template and primers. Hot start DNA Polymerase is activated by a 5 minutes incubation step at 95°C. This prevents extension of nonspecifically annealed primers and primer dimers formed at low temperatures during qPCR setup. The kit includes the components necessary for performing PCR amplification, and has been successfully used to amplify and detect a variety of DNA targets such as genomic DNA, cDNA and plasmid DNA.

KIT CONTENTS

Label	Volume
RealMOD™ Green W² 2x qPCR mix	1 ml

† Spin down before use

STORAGE AND STABILITY

- Storage condition : Store the product at -20℃
- RealMOD™ Green W² 2x qPCR mix are light-sensitive; avoid prolonged exposure to intense light.
- Expiration date : The solution is stable for 1 year from the date of shipping when stored and handled properly.

WIDE INSTRUMENT COMPATIBILITY

RealMOD™ Green W² 2x qPCR mix is designed for use with standard cycling mode on standard and fast qPCR platforms regardless of requirements in ROX. 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

PRODUCT WARRANTY AND SATISFACTION GUARANTEE

At iNtRON we pride ourselves on the quality and availability of our technical support. Our CRT center is staffed by experienced scientists with extensive practical and theoretical expertise in molecular biology and the use of iNtRON products. If a iNtRON product does not meet your expectations, simply call your local distributor. If you have questions about product specifications or performance, please call iNtRON Technical Services or your local distributor.

NOTICE BEFORE USE

The RealMOD™ Green W² 2x qPCR mix is intended for research use only. This product is not intended for the diagnosis, prevention, or treatment of disease. All due care and attention should be exercised in the handling of the products. Do not use internally or externally in humans or animals. Please observe general labolatory precaution and utilize safety while using this kit .

APPLICATIONS

- Real-time PCR : Detection and quantification of DNA and cDNA targets
- Gene expression profiling : Gene knockdown verification
- Microbial detection : Viral load determination
- Array validation : SNP genotyping

PROTOCOL

1. Thaw the RealMOD™ Green W² 2x qPCR mix, template DNA, primers and DNase/RNase free Water on ice. Mix each solution well.

2. Mix the reaction mix thoroughly, and centrifuge briefly to collect solutions at the bottom of PCR tubes or plates, and then store on ice protected from light.

Reagent	20 µl Reaction	50 µl Reaction	Final Concentration
RealMOD™ Green W² 2x qPCR 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
Template DNA	Variable*	Variable*	Variable*
DNase/RNase free Water	Up to 20 µl	Up to 50 µl	-

* Concentration of cDNA : 0.1 pg/µl – 10 ng/µl / Plasmid DNA < 50 ng / gDNA : 500 – 1000 ng/µl

3. Perform qPCR reactions using the following cycling program :

qPCR Steps	Temp.	Time	Cycle(s)
Initial activation*	95℃	10min*	1
Denaturation	95℃	20 sec	25 - 40
Annealing	50℃ - 65℃	40 sec	
Elongation	72℃	60 sec./kb	
Final Extension	72℃	5min.	1
Melting curve	Refer to specific guidelines for instrument used		

* To activate the polymerase, include an incubation step **at 95℃ for 10 minutes** at the beginning of the qPCR cycle.

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

5. After the reaction is completed, perform analysis.

GENERAL CONSIDERATION

1. Primer design guidelines
   The specific amplification, yield and overall efficiency of any Real-time PCR can be critically affected by the sequence and concentration of the primers, as well as by the amplicon length. We strongly recommend taking the following points into consideration when designing and running your Real-time PCR.
   1) Use primer-design software, such as Primer3 (http://frodo.wi.mit.edu/primer3/) or visual 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 four consecutive G residues.
   7) Make sure the five nucleotides at the 3' end contain no more than two G and/or C bases.

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.

TERMS USED IN REAL-TIMEPCR

Term	Definition
Baseline	The initial cycles of Real-time PCR in which there is little or no change in fluorescencesignal.
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.
Passivereference	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 detectortype).
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).

TROUBLE SHOOTING GUIDE

This troubleshooting guide may be helpful in solving problems that may frequently arise. The sci entists 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 productsignal in qPCR	
1) Pipetting error or agent	• Check the concentrations and storage conditions of the reagents, missing re including primers, template DNA. Repeat thePCR.
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	• Decrease annealing temperature in steps of 2℃.
5) Annealing temperature toohigh	• Increase annealing temperature in steps of 2℃.
6) Annealing temperatur e too low	• Reposition the sample tubes.
7) Incorrect setting for sample position.	• Confirm the data collection setting.
8) Incorrect setting for data collection	
Variation in detection	
1) Inappropriate concentratio n of primers	• Optimize primer concentration according to the instructions.
2) Failure or malfunction of e	• Check the device. devic
3) Variation of dispensed	• Increase the reaction volume. volume
4) Inappropriate cycle nditions	• Confirm Tm of the primers. co
Poor dynamic range of CTvalue	
1) Template amount too h igh	• Do not exceed maximum recommended amount of template. Do not use more than 500ng template.
2) Template amount too	• Increase template amount, if possible. low
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 PCRProducts	
1) Annealing too low	• Temperature increase annealing temperature in increments of 2℃.
	• Decrease the amount of primer.
2) To much amountof p rimer	

ORDERING INFORMATION

Product Name	Amount	Cat. No.
G-spin™ Total DNA Extraction Mini Kit	50 col.	17045
	200 col.	17046
HiSenScript™ RH(-) cDNA Synthesis Kit	50 rxn.	25014
G-spin™ Genomic DNA Extraction Kit (for Bacteria)	50 col.	17121
G-DEX™ IIc Genomic DNA Extraction Kit (Cell/Tissue)	300 T	17231
G-DEX™ IIb Genomic DNA Extraction Kit (For blood)	200 T	17241

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