RealMOD™ Green AP 5x qPCR mix

For Real-time quantitative PCR

Suitable for both ROX-dependent & ROX-independent qPCR cyclers

RUO *Research Use Only* REF *25348* Σ 250 -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 AP 5x qPCR mix is a 5x concentration premix type reagent, which contains all necessary reagents for Real-time PCR reaction except for primers and template DNA. Intercalating dye in the master mix enables the analysis of many different target genes. This product has the effect of suppressing primer-dimer formation, which is especially important matter in intercalating dye assay and this effect makes possible the accurate quantitative analysis in a wide range concentration of template DNA by minimizing non-specific amplification.

RealMOD™ Green AP 5x qPCR mix is an optimized ready-to-use solution for real time quantitative PCR assays, including EvaGreen® dye, and the solution is activated by 12 minutes incubation at 95℃. Hot-start mechanism prevents extension of non-specifically annealed primers and primer-dimer formation at low temperatures during qPCR setup.

KIT CONTENTS

Label	Volume
RealMOD™ Green AP 5x qPCR mix	1 ml

† Spin down before use

STORAGE AND STABILITY

- Storage condition : Store the product at -20℃
- RealMOD™ Green AP 5x qPCR mix are light-sensitive; avoid prolonged exposure to intense light.
- Shipping and temporary storage for up to 1 month at room temperature has no detrimental effects on the quality of RealMOD™ Green AP 5x qPCR mix.
- Expiration date : The solution is stable for 1 year from the date of shipping when stored and handled properly.

WIDE INSTRUMENT COMPATIBILITY

RealMOD™ Green AP 5x qPCR mix is designed for use with standard cycling mode on standard and fast qPCR platforms regardless of requirements in ROX. The Mix 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 OS 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 AP 5x 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.

BENEFITS

- Highly specific and reproducible real time PCR
- Excellent efficiency in case of low copy number targets
- Superior performance with long (up to 500 bp) and GC-rich templates
- Blue visualization dye for easy pipetting

APPLICATIONS

- Detection and quantification of targets DNA and cDNA
- Profiling gene expression
- Microbial detection
- Viral load determination

PROTOCOL

1. Thaw the RealMOD™ Green AP 5x 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	Final Concentration
RealMOD™ Green AP 5x qPCR mix	4 µl	1X
Forward Primer (10 µM)	0.2 – 0.4 µl	100 – 200 nM
Reverse Primer (10 µM)	0.2 – 0.4 µl	100 – 200 nM
Template DNA	Variable*	Variable*
DNase/RNase free Water	Up to 20 µl	-

* Concentration of cDNA : 0.1 pg/µl – 10 ng/µl / gDNA : 10 pg/µl – 4 ng/µl

3. Perform qPCR reactions using the following cycling program:

qPCR Steps	Temp.	Time	Cycle(s)
Initial activation*	95℃	12min*	1
Denaturation	95℃	15 sec	35 - 40
Annealing‡	60℃ - 65℃	20 – 30 sec‡	
Elongation‡	72℃	20 – 30 sec‡	
Melting curve	Refer to specific guidelines for instrument used		

* To activate the polymerase, include an incubation step **at 95℃ for 12 minutes** at the beginning of the qPCR cycle.
‡ Use 20 sec for annealing and elongation for templates shorter than 150 bp.

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.

EvaGreen®Dye

EvaGreen® is a DNA-binding dye with many features that make it a superior alternative to SYBR® Green I for qPCR. Apart from having similar spectra, EvaGreen® has three important features that set it apart from SYBR® Green I. EvaGreen® shows much less PCR inhibition, and is an extremely stable dye. Furthermore it has been shown to be nonmutagenic and noncytotoxic. EvaGreen® is compatible with all common real-time PCR cyclers – simply select the standard settings for SYBR® Green or FAM.

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-TIME PCR

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).

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 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. If necessary, make new serial dilutions of template DNA from the stock solutions. Repeat the PCR using the new dilutions.
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 of device.
3) Variation of dispensed volume	• Increase the reaction volume.
4) Inappropriate cycles 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. Do not use more than 500ng 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) Annealing too low	• Temperature increase annealing temperature in increments of 2℃.
2) To much amount of primer	• Decrease the amount of primer.

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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