# RealMOD™ Probe W² 2x qPCR mix

For Real-time quantitative PCR

RUO *Research Use Only* REF *25351* 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™ Probe W² 2x qPCR mix is an optimized ready-to-use solution for real-time quantitative PCR assays. It comprises all the components necessary to perform qPCR: Taq DNA Polymerase, dNTPs, $MgCl_2$ and PCR buffer, except DNA template and primers. 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™ Probe W² 2x qPCR mix | 1 ml |

† 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 and handled properly.

## WIDE INSTRUMENT COMPATIBILITY

RealMOD™ Probe W² 2x qPCR mix 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

## APPLICATIONS

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

## NOTICE BEFORE USE

The RealMOD™ Probe 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.

## USE OF THE ROX REFERENCE DYE

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

## PROTOCOL

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

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

**3. The following table shows recommended component volumes:**

| Component | 20 µl Reaction | 50 µl Reaction | Final Concentration |
|---|---|---|---|
| RealMOD™ Probe 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 |
| Fluorescence Probe | Variable | Variable | Variable |
| Template DNA | ≥ 1 µl | ≥ 1 µl | As needed |
| 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

**4. Perform qPCR reactions using the following cycling program :**

| qPCR Steps | Temp. | Time | Cycle(s) |
|---|---|---|---|
| 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 |

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

**5. Place the PCR tubes or plates in the Real-time cycler, and start the cycling program.**
**Note : 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 and, increase extension time to generate longer PCR products.**

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

## 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 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 infor mation 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 g reagent | • Check the concentrations and storage conditions of the reagents, missin 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 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 qPCR 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 e | • Check the device. devic |
| 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 h igh | • 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 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 | |

## ORDERING INFORMATION

| Product Name | Amount | Cat. No. |
|---|---|---|
| G-spin™ Total DNA Extraction Mini Kit | 50 col. | 17045 |
| | 200 col. | 17046 |
| FC cDNA Synthesis Kit (Fast & Clear) | 25 rxn. | 25088 |
| 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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