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RealTime GreenR™ PCR Master Mix 2X

The technology of the dye used in this product is jointly developed by CSIR-CDRI and Biotech Desk Pvt. Ltd.

Patent #: IN202211040311

Cat # : ☐ PCR013, ☐ PCR013S
Pack Size: ☐ 2.5 mL; ☐ 250µL
Storage: -20°C

CSIR-Central Drug Research Institute

Introduction

G2P Real-time GreenR™ PCR 2X Master Mix is suitable for routine and highly sensitive qPCR experiments. G2P GreenR™ PCR 2X contains all the components required for a successful polymerase chain reaction, which includes appropriate amounts of dH_2O, DNA polymerase, dNTPs, $MgCl_2$, and reaction buffers for effective amplification. The kit contains a new dye called GreenR™ which is more sensitive than conventional SYBR Green. The 2X premix is capable of detecting a wide range of DNA (100bp-1Kb) in real-time PCR. It can be used in all Real-time PCR machines available and maintains specificity and sensitivity of the reaction.

Kit Content

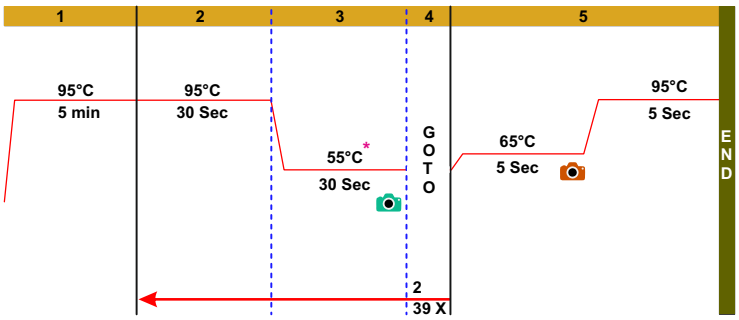
G2P Real-time GreenR™ PCR 2x Master Mix	: 2.5 mL (500µL x 5)
ROX Solution (25 µM)	: 100µl (only for ABI systems)
DEPC water	: 1.25 mL X 2

PCR set up and Amplification Guide

PCR reaction setup

Component	Amount per reaction (µl)	Amount per reaction (µl)	Final concentration
Real Time GreenR™ PCR Master Mix 2X	25	12.5	1X
Template	Variable	Variable	1 to 100 ng in 5µl-10µl
Forward Primer (5µM)	0.5 - 9	0.2 - 3.6	50 to 900 nM
Reverse Primer (5µM)	0.5 - 9	0.2 - 3.6	50 to 900 nM
Distilled Water (dH_2O)	Add to 50	Add to 25	-
Total Reaction Volume	50	25	-

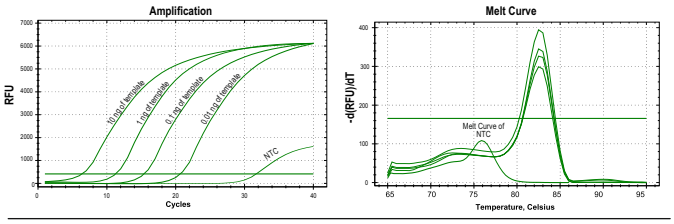
General PCR Condition using Bio-Rad CFX96™



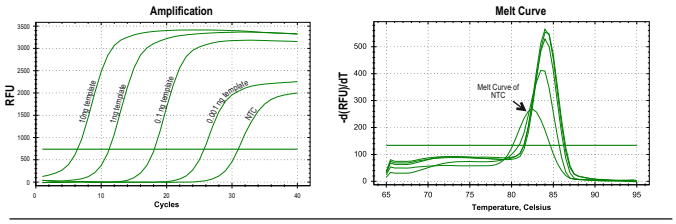
** Depends on the primer Tm and template used (Need to be optimized)*
Initial denaturation at 95°C for 5 min is required to activate the polymerase used in this master mix

Comparison of G2P Real-time GreenR™ PCR 2X Master Mix with Takara SYBR® Premix Ex TaqII™

Amplification curve comparison

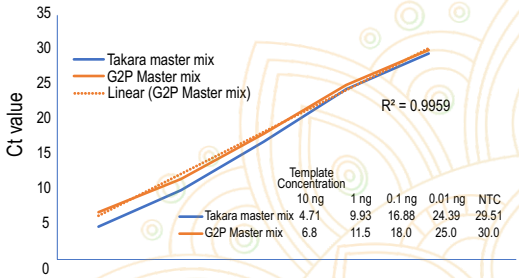


Real Time GreenR PCR Master Mix 2X PCR



Takara Green Premix Ex Taq II

Correlation



Troubleshooting

Gene expression quantitation:

Relative quantitation: Target gene expression is measured against an internal control. Gene expression can be measured by the quantitation of cDNA converted from mRNA corresponding to this gene relative to a calibrator sample serving as a physiological reference. In a typical experiment, gene expression levels are studied as a function of either a treatment of cells in culture, of patients, or of tissue type. The calibrator sample in each case is the cDNA from either the untreated cells or patients, or a specific tissue type. All quantitation should be normalized to an endogenous control such as 18S rRNA to account for variability in the (i) initial concentration of RNA (ii) quality of total RNA and (iii) conversion efficiency of the reverse transcription reaction.

Set C_tA1 as the C_t value of the target gene of sample 1, and C_tB1 as the C_t value of the internal control gene of sample 1; Set C_tA2 as the C_t value of the target gene of sample 2, and C_tB2 as the C_t value of the internal control gene of sample 2. The expression difference (in fold) of the target gene in sample 1 Vs sample 2 can be calculated as follows :

$$\Delta\Delta C_t = (C_tA2 - C_tB2) - (C_tA1 - C_tB1)$$

The expression level of the target gene in sample 2 would be $2^{-\Delta\Delta C_t}$ times that of sample 1.

Absolute quantitation: Compares the C_t value of an unknown sample against a standard curve with known copy numbers. Absolute quantitation is applicable only if the isolation procedure and sample contents do not affect PCR amplification. The quantitation of genomic DNA may lend itself to absolute quantitation against a standard curve.

Passive reference ROX:

ROX is a dye molecule included in the SYBR Green PCR Master Mix that does not participate in the PCR amplification. On Applied Biosystems real-time PCR systems, the passive reference provides an internal reference to which the SYBR Green / dsDNA complex signal can be normalized during data analysis. Normalization is necessary to correct for well-to-well fluorescent fluctuation. Few PCR machine does not require the use of any passive reference dye. please refer the user manual of the PCR used.

ROX reference dye required in some qPCR machines. Please refer the consult the instrument manual for amount of ROX dye to be used.

Determination of baseline and threshold:

Please refer to the real-time PCR system software used to calculate baseline and threshold values for a detector or manually set it up according to amplification curves.

Troubleshooting

Rough amplification plot: It is caused by system rectification due to weak signal. Elevate the template concentration and repeat the reaction.

Broken or downward amplification plot: The concentration of templates is too high. The end value of the baseline is bigger than Ct value. Decrease the end of the baseline C_t value - 4), and re-analyze the data.

Amplification plot goes downward suddenly: There are bubbles left in the reaction tube, which break up when the temperature rises, thus the instrument detects the sudden decrease of the fluorescence value. Spin briefly and check closely if there are bubbles left before the reaction.

No amplification plot:

- Cycling number is insufficient: Generally, the cycling number is set around 40. But notice that too many cycles will result in the excessive background, thereby reducing the reliability of the data.
- Check if there is signal collection procedure during cycling: in two-step program, the signal collection is usually positioned at the annealing and extension stage; for the three-step program, the signal collection should be positioned at 72 °C extension stage.
- Check if the primers are degraded: Test the integrity of primers after long-term storage through PAGE electrophoresis to confirm the presence of the primers in the solution.
- The concentration of templates is too low: Reduce the dilution and retry. For target gene with unknown expression level, begin without template dilution.
- Degradation of templates: Prepare new templates and retry.

C_t Value too high

- Low amplification efficiency: Optimize the reaction. Try three-step program or re-design primers.
- The concentration of templates is too low: Reduce the dilution and retry. For target gene with unknown expression level, begin without template dilution.
- Degradation of templates: Prepare new templates and retry.
- The amplicon is too long: The length of the amplicon is recommended to be within 100 bp - 300 bp.
- There are PCR inhibitors in the reaction: They are usually brought in when adding templates. Increase the dilution folds or prepare new templates and retry.

Apparent amplification can be observed in negative control

- The reagents or water used is contaminated: Change to new reagents or water and retry. The reaction should be set up in a clean bench to minimize contamination from the air.
- Appearance of primer dimer: It is normal that amplification occurs in negative control after 30-35 cycles. Analysis can be performed combining with the melt curve to confirm.

Pre-denaturation / enzyme activation time

- The reagents or water used is contaminated: Change to new reagents or water and retry. The reaction should be set up in a clean bench to minimize contamination from the air.
- This mix is based on a hot-start DNA polymerase, the pre-denaturation / enzyme activation condition should be set to 95 °C for 5 minutes to thoroughly activate the enzyme. If the template is GC-rich, the pre-denaturation / enzyme activation time should be prolonged to 10 minutes.

Note:

In NTC, the C_t value > 28 maybe observed because of primer dimer formation.

The suggested template amount is 10-100 ng for genomic DNA or 1-10 ng for cDNA and plasmid templates.

Use of ROX dye is optional for the PCR machine used. Please see the manufacture manual for the amount of ROX dye used