Protocol

TD-P Revision 1.0

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Quantitative Polymerase Chain Reaction (qPCR)

Utilizing qPCR Master Mix with SYBR® Green

Introduction

Our qPCR Master Mix with SYBR® Green is a ready-to-use cocktail containing all components except primers and template, for the amplification and detection of DNA in qPCR. The qPCR Master Mix with SYBR® Green contains integrated chemically-modified hot start Taq DNA polymerase, SYBR® Green I fluorescent dye, ROX dye, $MgCl_2$, dNTPs and stabilizers. This master mix is ideal for high-throughput real-time PCR screening and validation. In addition, the amplification step features a high quality hot start Taq DNA polymerase, which offers higher fidelity and better amplification.

GoldBio's qPCR Master Mix with SYBR® Green can be used for gene expression validation, absolute gene expression quantification, mutation, pathogen and viral detection, genetically modified organisms (GMOs) characterization and genetic profiling. The advantages of this master mix includes enhanced efficiency, specificity and sensitivity, compatibility with all real-time PCR instruments, superior gene expression results under various cycling conditions, and robust and active cDNA amplification at temperatures up to 55°C. Here, we describe a general protocol for the use qPCR Master Mix with SYBR® Green in qPCR.

Materials

- qPCR Master Mix with SYBR® Green (GoldBio Catalog # M-915)

Not included:

- H_2O PCR grade
- Reverse and forward primers

Storage and Handling

- Store qPCR Master Mix with SYBR® Green at -20°C.
- This product may be shipped on blue ice and should be stored at -20°C immediately upon arrival. When stored under the recommended conditions and handled correctly, this product should be stable for at least 1 year from the date of receipt.

Method

1. Place kit components, primers and cDNA samples on ice.

2. Mix and then centrifuge briefly to collect contents at the bottom of the tube.

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3. Prepare a master mix for each reaction and control plus 10% extra to allow for pipetting error according to the following table:

PCR reaction set up:	
Component	**Volume**
Diluted cDNA	1-5 µl
Forward primer (5µM)	1.0 µl
Reverse primer (5µM)	1.0 µl
qPCR Master Mix with SYBR® Green	10.0 µl
H_2O up to	20.0 µl

4. Mix the reaction mixture thoroughly.

5. Program the thermal cycler according to the manufacturer's instructions.

6. A typical PCR cycling program is outlined in the following table.

PCR cycling conditions:			
Steps	**Temperature**	**Time**	**Cycles**
Initial denaturation	95°C	15 min	1
Denaturation	95°C	5 sec	30-40
Annealing/Extension	~60°C	30 sec	
Melting curve analysis	According to instrument guidelines		

Note: For the annealing/extension step, anneal at optimal annealing temperature for 30 seconds followed by the minimum time required for data acquisition at 72°C according to instrument guidelines.

7. Place the PCR tubes in the thermal cycler and start the cycling program.

8. Analyze the data according to the manufacturer's protocol.

Associated Products

- dNTP Mix (GoldBio Catalog # D-900)

- RT-PCR Kit (GoldBio Catalog # R-920)
- RT-qPCR Kit (GoldBio Catalog # R-925)
- One Step RT-qPCR Kit (GoldBio Catalog # R-915)
- First Strand cDNA Synthesis Kit (GoldBio Catalog # D-925)
- Reverse Transcriptase (GoldBio Catalog # R-900)
- qPCR Master Mix with SYBR® Green (GoldBio Catalog # M-915)
- Goof-Proof™ qPCR Master Mix (GoldBio Catalog # G-700)
- Goof-Proof™ qPCR Master Mix with ROX (GoldBio Catalog # G-705)
- Goof-Proof™ Universal Probe Master Mix (GoldBio Catalog # G-710)
- Goof-Proof™ Universal Probe Master Mix with ROX (GoldBio Catalog # G-715)
- EvaGreen® Dye, 2000x in DMSO (GoldBio Catalog # E-675)
- EvaGreen® Dye, 20x in Water (GoldBio Catalog # E-670)