Cat. No. AERM-1208

For Research Use Only

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REAL AZMA

SYBR GREEN qPCR Master Mix Without ROX

Introduction

REAL AZMA SYBR GREEN qPCR master mix is a reaction mix containing all components, with the exception of template and primers. It is optimized for real-time quantitative PCR assays. It combines the hot-start technology of Taq DNA polymerase and SYBR Green I fluorescent dye. The Master Mix is supplied at a 2 X concentration and contains Taq DNA polymerase, SYBR Green I dye, $MgCl_2$, dNTPs and stabilizers. In addition, the amplification step features a high-quality hot start Taq DNA polymerase, which offers higher fidelity and better amplification.

REAL AZMA SYBR GREEN qPCR Master Mix 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 include enhanced efficiency, specificity and sensitivity, compatibility with all real time PCR instruments, superior gene expression results under various cycling conditions.

Maximum Flexibility and Convenience

- REAL AZMA SYBR GREEN qPCR master mix provides maximum flexibility at reduced cost because no target-specific TaqMan™ probes are required
- SYBR Green I dye is a double-stranded DNA binding dye that detects any double-stranded DNA generated during PCR
- The hot-start DNA Polymerase enzyme minimizes nonspecific product formation (including primer-dimers), yielding superior performance and sensitivity
- Passive Internal Reference 1 is provided to normalize non-PCR—related fluorescence fluctuations
- This minimizes well-to-well variability that can result from a variety of causes, such as pipetting error or sample evaporation
- SYBR Green I dye is ideal for target identification (screening assays) or when a limited number of assays is needed.

Precaution

- Store extracted DNA material away from master mix and add it to the reaction mix in a separate area.
- Use disposable gloves, laboratory coats and eye protection while handling samples and reagents. Thoroughly wash hands afterwards.
- Thaw master mix and primers thoroughly on ice before starting experiment.
- Clean and disinfect all sample or reagent spills using a disinfectant such as 0.5% sodium hypochlorite, or other suitable disinfectant.
- Avoid contact of specimens and reagents with the skin, eyes and mucosa.
- If skin, eyes and mucosa contact immediately flush with water, seek medical attention.
- Use of this product should be limited to personnel trained in the techniques of DNA amplification.

Materials

- qPCR Master Mix with SYBR Green (REAL AZMA Catalog # AERM 1208)
  Not included:
- H2O 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 production.

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

Recommended PCR reaction set up

Component	Volume
2X qPCR Master Mix	10.0 µl
10 µM Forward Primer	0.25 µl
10 µM Reverse Primer	0.25 µl
PCR Grade DDW	9 µl
Template (50 ng/ µl)	0.5 µl
Total	20 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.
7. Place the PCR tubes in the thermal cycler and start the cycling program.
8. Analyze the data according to the manufacturer's protocol.

PCR cycling conditions

Steps	Temperature (°C)	Time	Cycles
Initial denaturation*	95	10 min	1
Denaturation	95	10 s	40
Annealing/Extension	~60°C	1 min	
Melting curve	According to instrument guidelines		

*This step also is necessary for enzyme activation.