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Safe-Green™ 2X PCR Taq MasterMix

Store at -20°C

Cat. No.	Product Name	Quantity
G472	Safe-Green™ 2X PCR Taq MasterMix	5 ml (200 PCR reactions)

Product Description

The Safe-Green™ 2X PCR Taq MasterMix is a ready-to-use mixture of high quality Taq DNA Polymerase, deoxynucleotides, and reaction buffer in a 2X concentration. It contains all the necessary reagents for the amplification of DNA templates as well as all essential elements required for downstream electrophoresis, completely eliminating the needs for EB and other gel staining dyes which are of significant environment concerns. To set up a PCR reaction: simply add DNA template, primers and ddH_2O. Up to 6 kb templates can be amplified with a single base (A) overhang at the 3'-end of PCR products.

Features and Benefits

- Completely eliminating DNA staining and running dyes in both gel and electrophoresis buffer.
- The simplest PCR formulation on earth.

Shipping and Storage

Keep at –20°C for long term storage and at 4°C for 3 months for daily use.

Protocol

PCR reactions should be assembled in a nuclease-free environment. DNA sample preparation, reaction mixture assemblage and the PCR process, in addition to the subsequent reaction analysis, should be performed in separate areas. The use of "clean", automatic pipettors designated for PCR and aerosol resistant barrier tips are recommended. Always keep the control DNA and other templates to be amplified isolated from the other components.

A control reaction, omitting template DNA, should always be performed to confirm the absence of contamination.

1. Add the following components to a sterile 0.2 ml PCR tube sitting on ice:

Components	Volume	Final Concentration
Template DNA	~100 ng	~2 ng/µl
Forward primer (10 µM)	1 - 2 µl	0.2 - 0.4 µM
Reverse primer (10 µM)	1 - 2 µl	0.2 - 0.4 µM
Safe-Green™ 2X PCR Taq MasterMix	25 µl	1X
Nuclease-free H_2O	up to 50 µl	-

Note: We recommend preparing a pre-mix for multiple reactions to minimize reagent loss and enable accurate pipetting.

2. Mix contents of tube and centrifuge briefly.
3. Incubate tube in a thermal cycler at 94°C for 3 mins to completely denature the template.
4. Perform 30-40 cycles of PCR amplification as follows:

   Denature: 94°C for 30 sec

   Anneal: 45°C - 72°C for 30 sec

   Extend: 72°C for 1 min/1 kb template

5. After additional elongation at 72°C for 5 mins, the amplified PCR products are ready for direct gel electrophoresis *(no running and staining dyes in both running buffer and gel!)* and to be stored at -20°C until use.
6. When performing gel electrophoresis, a specially formulated DNA markers have to be used as there is no DNA staining dye in both gel and running buffer.
7. It is strongly recommended to use the following DNA markers to analyze the PCR product:
   Safe-Green™ 100 bp Opti-DNA Marker (Cat. No. **G473**)
   Safe-Green™ 1 kb Opti-DNA Marker (Cat. No. **G474**)