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2× LiTaq™ HotStart PCR Master Mix

Cat. #: M0021 Size: 5 ml

Introduction

LiTaq™ HotStart PCR Master Mix is a 2× concentrated solution of HotStart Taq DNA Polymerase, dNTPs, and all of the components required for PCR, except DNA template and primers. HotStart Taq PCR Master Mix provides robust and reliable performance in PCR amplification. This pre-mixed formulation saves time and reduces contamination due to a reduced number of pipetting steps required for PCR set up. HotStart Taq PCR Master Mix contains inert, non-toxic dyes to visualize PCR mixing step, and also allows direct loading of PCR products on to gels for electrophoresis.

The HotStart Taq DNA Polymerase is a chemically modified Taq DNA Polymerase, whose enzyme activities can only be activated after 3-5 minutes of incubation at 95°C. The HotStart Taq Polymerase uses amplification conditions for regular Taq DNA Polymerase, except no polymerase activity will be present before the onset of thermal cycling. This prevents nonspecific DNA amplification and primer dimer formation.

Package Information

Components	M0021
2× LiTaq™ HotStart PCR Master Mix	5 ml

Storage

All materials should be stored at -20°C.

Protocol

The following basic protocol serves as a general guideline and a starting point for any PCR amplification. Optimal reaction conditions (incubation times and temperatures, concentration of HotStart Taq DNA polymerase, primers, Mg^{2+}, and template DNA) vary and need to be optimized. Critical parameters and troubleshooting information are documented in reference 1.

Assemble PCR reactions in a nuclease-free environment. Use of "clean" dedicated pipettes and aerosol resistant barrier tips are recommended.

1. Thaw template DNA and all reagents on ice. Mix each solution by vortexing, and centrifuge briefly to collect residual liquid from the sides of the tubes.

2. Prepare the following reaction mixture in a PCR tube on ice:

Components	Reaction Volume
2× LiTaq™ HotStart PCR Master Mix	25 µl
DNA template (< 250 ng/50 µl)	variable
Upstream primer (10 µM)	2.5 µl
Downstream primer (10 µM)	2.5 µl
Distilled water (dH_2O)	To 50 µl
Total reaction volume	50 µl

3. Mix, and then briefly centrifuge the contents.

4. Perform PCR using the recommended thermal cycling conditions outlined below:

Step	Temperature	Time	Cycle
Initialization	95°C	1 - 3 min	1
Denaturation	95°C	30 sec	25-35
Annealing	Tm-5	30 sec	
Extension	72°C	1 kb/min	
Final Extension	72°C	10 min	1
Holding	4°C	-	1

5. Analyze the amplification products by agarose gel electrophoresis.

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