LIFESCT
Life Scientists' Service Center
Email: support@lifesct.com
Tel: +1-301-890-6420 Fax: +1-240-252-7376
9610 Medical Center Drive, Rockville, MD 20850, USA

2× LiTaq™ Fast PCR Master Mix

Cat. #: M0020 Size: 5 ml/20 ml

Introduction

2× LiTaq™ Fast PCR Master Mix is an optimized ready-to-use solution containing HotStart DNA polymerase screened by gene modification with high success rate. This product has extremely high DNA affinity and PCR processivity, enabling rapid amplification at an extension rate of 1-30 sec/kb (<2 kb, 1 sec/kb; <5 kb, 5 sec/kb; <10 kb, 10 sec/kb). it boasts wide template compatibility, suitable for genomic DNA or crude templates from animals, plants, bacteria, *etc*., as well as target sequences with high or low GC content. In addition, this pre-prepared 2× Premix only requires the addition of primers and templates for amplification. it is pre-mixed with electrophoresis indicators, allowing the PCR products to be directly used for electrophoresis, which is convenient and efficient.

Package Information

Components	M0020-05	M0020-50
2× LiTaq™ Fast PCR Master Mix	5×1 ml	20×1 ml

Storage

All materials should be stored at -20°C.

Protocol

1. General reaction mixture for PCR:

Components	Reaction Volume
LiTaq™ Fast PCR Master Mix	25 μl
Template DNA*	Optional
25× Primer Mix**	2 μl
ddH_2O	to 50 μl

* Due to the different copy numbers of target gene contained in templates of different species, the template canbe diluted in gradient to determine the optimal template usage. The volume of crude template must not exceed10% of the PCR reaction volume.

** Typically 0.2 μM primers concentration yields good results; if the performance is poor, the primerconcentration can be adjusted within the range of 0.2-1 μM.

2. Thermocycling conditions for a routine PCR:

Step	Temperature	Time	Cycle
Initialization	95°C	3~5 min	1
Denaturation	95°C	10~15 sec	30~35
Annealing	55-65°C*	10~30 sec	
Extension	68-72°C	X sec**	
Final Extension	72°C	3~5min	1

* Annealing temperature should be set based on primer Tm. Optimize using a temperature gradient if necessary.Increase temperature if nonspecific amplification occurs.

** Extension time guidelines: <2 kb: 1 sec/kb; <5 kb: 5 sec/kb; <10 kb: 10 sec/kb. Adjust based on actual fragment length.

Handling Notes

1. Mix well before use and avoid repeated freeze-thaw cycles.
2. Perform initial denaturation at 95°C or 98°C for 3-5 minutes for HotStart activation.
3. The fast DNA polymerase demonstrates an extension rate up to 1 sec/kb.
4. To increase PCR product yield, appropriately extending the elongation time is recommended.
5. This product is compatible with multiplex amplification of fragments <10 kb, with recommended extension time of 30 sec/kb for multiplex PCR.
6. The system exhibits strong adaptability and is suitable for rapid amplification reactions.
7. Suitable for applications including fast PCR, genotyping, and colony PCR.
8. For crude template preparation from animal/plant tissues, thorough mincing of tissues is recommended to ensure complete DNA release during lysis.
9. When amplification from crude templates is unsatisfactory, appropriate template dilution prior to PCR may improve results.

Troubleshotting

Non-specific bands or smeared bands are observed

1. Primer design optimization is recommended.
2. Increasing the annealing temperature or reducing primer concentration is suggested.
3. Using purified templates is advised.

Target bands appear in the negative control

Repeat the experiment with fresh mix, primers, or nuclease-free water. Prepare the reaction mix in a biosafe tycabinet or laminar flow hood to minimize aerosol contamination.

Target bands are faint or absent

1. Optimize primer design.
2. Slightly increase template input.
3. Verify primer integrity and template quality (degradation may occur); repeat the experiment with newly prepared templates if necessary.
4. Ensure the reaction system and amplification program are correctly set up.

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