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2× LiTaq™ Ultra HiFi PCR Master Mix

Cat. #: M0031 Size: 1 ml/5 ml

Introduction

2× LiTaq™ Ultra HiFi PCR Master Mix is a ready-to-use high fidelity PCR premix, It adopts a genetically engineered hot-start high fidelity DNA polymerase, which has extremely high DNA affinity and processivity. It is well compatible with complex templates and partially degraded templates, and its fidelity is about 154 times that of standard Taq polymerase. The reagent contains unique extension factors and specificity-promoting factors, which greatly improve the long-fragment amplification ability, amplification specificity and amplification yield.

This product contains hot-start high fidelity DNA polymerase, dNTPs and an optimized buffer system. Only primers and templates need to be added for amplification, which reduces pipetting operations, improves detection throughput and result reproducibility; this product also has a version containing electrophoresis indicators, which can be directly loaded for electrophoresis after PCR reaction. It is optimized for experiments such as gene cluster cloning and ultra-long fragment assembly.

Package Information

Components	M0031-01	M0031-05
2× LiTaq™ Ultra HiFi PCR Master Mix	1 ml	5 ml

Storage

All materials should be stored at -20°C.

Handling Notes

1. Do not use dUTP and primers and templates with uracil.

2. The DNA polymerase is modified with monoclonal antibodies, The reaction system can be prepared at room temperature, but it is recommended to place it on ice before use and store it in the refrigerator immediately afteruse.

3. When amplifving fragments >10kb, it is recommended that the primer length is >26nt, and the primer designshould meet general principles.

Protocol

1. General reaction mixture for PCR:

Components	Reaction Volume
2× LiTaq™ Ultra HiFi PCR Master Mix	25 μl
Template DNA*	Optional
Primer 1	1 μl (0.1-0.4 μM**)
Primer 2	1 μl (0.1-0.4 μM**)
ddH_2O	to 50 μl

* The optimal reaction concentration varies for different templates. The recommended template usage for 50 ul system is as follows:

Genomic DNA	50~400 ng
Plasmid or viral DNA	10 pg~30 ng
cDNA	1~5 μl
Crude template***	1~2 μl

** In general, the final concentration of primers can be 0.2 μM. When non-specific amplification occurs.reducing the final concentration of primers to 0.1 μM can improve specificity.

*** For whole blood samples, it is recommended to add 1%-2% of whole blood template to the PCR reaction system. The initial denaturation time in the PCR program needs to be adjusted to 5min. After the PCR reaction, centrifuge at 4000 rpm for 1 min to precipitate blood cell debris, and take the supernatant for downstream analysis; for fungi, bacteria, animal and plant tissue samples, it is recommended to use lysis buffer for cell lysis before PCR reaction.

Gently pipette to mix, and centrifuge instantaneously to collect the reaction solution on the tube wall to the bottom of the tube.

2. Thermocycling Conditions for PCR

Step	Temperature	Time	Cycle
Initialization	98°C	30 sec	1
Denaturation	98°C	15 sec	25-35
Annealing	56-72°C	15 sec	
Extension	72°C	5~15 sec/kb	
Final Extension	72°C	3~5 min	1
Holding	4°C	-	1

* For most templates, initial denaturation is set to 30 sec. if the GC content of the template is too hign, it can be extended to 1 min.

** Please set the annealing temperature according to the Tm value of the primer. If the Tm value ofthe primelis relatively high, the annealing step can be deleted, and a 2-step PCR amplification program can be used (when using the two-step method, the annealing and extension temperature can be optimized between 65°C-68°C). If necessary, a temperature gradient can be established to find the optimal temperature for the combination of primers and templates. In addition, the annealing temperature directly determines the amplification specificity. If the amplification specificity is found to be poor, the annealing temperature can be appropriately increased.

Note: When amplifying target fragments below 5 kb, the extension time can be set to 5 sec/kb; when amplifying target fragments of 5-15 kb, the extension time can be set to 10 sec/kb: when amplifying target fragments above 15kb, the extension time can be set to 15 sec/kb; if the concentration after amplification is low, the primer concentration or extension time can be appropriately increased.

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