2X High Fidelity PCR Mastermix

Cat. No.	Product Description	Size
HFTP01-01	2x High Fidelity PCR Mastermix, Blue	5x1 ml; 400 RXN
HFTP01-02	2x High Fidelity PCR Mastermix, Blue	20x1 ml; 1600 RXN

Product Description

This product is a premixed system composed of efficient high fidelity PCR Master Mix, Mg2+, dNTPs, PCR stabilizers and enhancers, with a concentration of 2x. High Fidelity PCR Master Mix is a hot start enzyme modified by antibodies that can perform high specificity Hot Start PCR. This enzyme exhibits both 5 '-3' polymerase activity and 3 '-5' exonuclease activity, with a fidelity approximately 52 times that of Taq DNA Polymerase and an amplification length of up to 20 kb.

In addition, this product uses a high stress resistant buffer and exhibits excellent performance in the amplification of crude samples, which can effectively amplify crude samples containing multiple PCR inhibitors that are difficult to amplify with ordinary PCR enzymes.

This product contains electrophoresis dye, and agarose gel electrophoresis can be carried out directly after PCR reaction.

Features

1. **High stress resistance**: The amplification ability of crude extracted animal and plant tissues is very strong. For samples such as blood and microorganisms, there is no need to extract nucleic acid, and sufficient amplification products can be obtained by directly adding them to the reaction solution.
2. **High amplification ability**: For short fragments and purified templates, the extension speed can reach 10-15 seconds/kb; For templates with longer or more complex fragments, it is recommended to use an extension speed of 30 seconds/kb; At the same time, it can also effectively amplify trace templates.
3. **High specificity:** Efficient hot start enzymes significantly improve amplification specificity.
4. **High-fidelity:** The fidelity of this product is approximately 52 times that of Taq DNA Polymerase. Applicable to experiments where PCR products are cloned on a vector and then sequenced.
5. **High stability:** Place this product at 37°C for accelerated stability testing for 10 days without affecting its performance.

Storage

Keep at –20°C.

Protocol

1. **Preparation of PCR reaction solution**

   Before preparing the reaction solution, please thoroughly mix all reagents. Frozen reagents should be completely thawed

Components	Volume	Final Concentration
Template DNA	~50 ng	~1 ng/μl
Forward primer (10 μM)	0.5-1 μl	200-400 nM
Reverse primer (10 μM)	0.5-1 μl	200-400 nM
2x High Fidelity PCR Mastermix, Blue	12.5 μl	1x
Nuclease-free H_2O	up to 25 μl	

   Note:1) All components can be proportionally scaled up or down, 2) After adding all liquids, please mix thoroughly before proceeding with PCR.

2. **Template**

   a. When using purified templates (PCR Reaction volume 25 μl)

Template type	Reference amount	Template amount
Genomic DNA from eukaryotes	5-250ng	100ng
Genomic DNA from prokaryotes	0.1-50ng	10ng
Plasmid DNA	10pg-25ng	1ng
cDNA	<100ng (RNA equivalent)	50ng (RNA equivalent)
λDNA	10pg-5ng	1ng

Note:

1) When a large amount of RNA is mixed into the amplification system, it will inhibit the PCR reaction: the length and purity of the template will have a significant impact on the PCR results. When the template quantity is sufficient, it is recommended to confirm the quality of the template by electrophoresis; When using reverse transcription reaction solution as a template, the amount of RNA added to 50uL PCR reaction solution should be controlled below 200ng.
2) Do not use templates containing uracil.

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b. When using raw material templates (PCR Reaction volume 50 µl)

Template type	Template Amount	
Escherichia coli liquid	Add 1-5µL	When stable amplification cannot be obtained, ddH2O can be used to dilute the bacterial solution appropriately.
Escherichia coli colony	Pick a small colony	
Yeast	Pick a small colony	
Filamentous bacteria	Pick a small colony	
Cell	10^1-10^5	
Blood	1-2µL	
Plant leaves	5-10mg	After the animal and plant samples are lysed with lysis solution, the supernatant is centrifuged and used as a template to avoid adding too many impurities to the PCR reaction solution.
Mouse tail	About 1-3mm	
Mouse toes	About 1-3mm	
Mouse ears	2-5mm^2 small pieces	

3. PCR cycle conditions

Step	Temperature	Time	Cycles
Pre denaturation	95℃	2min	1
Denaturation [1)] Annealing [2)]	95℃ Determine based on primer Tm	15sec 20sec	30-35cycles[4)]
Extension [3)]	72℃	10-15sec/kb	
Final extension	72℃	5min	1

Note

1) Denaturation: The pre denaturation time for simple templates is set at 95 ℃ for 30 seconds to 1 minute. For complex templates, the pre denaturation time can be extended to 3 minutes.
2) Annealing: The annealing temperature is adjusted according to the theoretical Tm value of the primer. Generally, in experiments, the annealing temperature is 3-5 ℃ lower than the Tm value of the primer. If the ideal amplification efficiency cannot be achieved, the annealing temperature should be gradually changed for optimization; When non-specific reactions occur, increase the annealing temperature appropriately.
3) Extension: Different templates have different extension time settings. When amplifying crude samples, please set it at 30sec/kb; When amplifying purified DNA and plasma, 10-15 seconds/kb can be fully amplified.
4) Cycle count: When the number of copies of the target fragment is small, it is recommended to increase the loop count to 35-45 cycles.

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Bioland Scientific LLC, Tel: (562) 602-8882, Fax: (562) 733-6008
Email: order@bioland-sci.com www.bioland-sci.com