PCR Plus Master Mix,Dye

Product Number: PCM42

Shipping and Storage

-20℃。

Components

Component	PCM42
2×PCR Plus Master Mix,Dye	5×1mL
ddH_2O	5×1mL

Description

This product is a premixed system composed of efficient PCR Plus Master Mix, Mg2+, dNTPs, PCR stabilizers and enhancers, with a concentration of 2×. PCR Plus 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 indicator, 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℃ for accelerated stability testing for 10 days without affecting its performance.

Protocol

1. Preparation of PCR reaction solution

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

Component	25μL reaction system	50μL reaction system	Final concentration
2×PCR Plus Master Mix,Dye(Dye)	12.5μL	25μL	1×
Forward Primer,10μM	0.5-1μL	1-2μL	0.2-0.4μM

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Reverse Primer,10μM	0.5-1μL	1-2μL	0.2-0.4μM
Template DNA in moderation	XμL	XμL	<250ng/25μL
ddH2O	Up to 25μL	Up to 50μL	/

Note:1)All components can be proportionally scaled up or down, and the primer concentration and template quantity can be adjusted appropriately according to needs.

2)After adding all liquids, please mix thoroughly before proceeding with PCR.

2. Template

2.1. When using purified templates, cDNA templates, etc., please refer to the following data for the amount added (PCR reaction solution is 25μL).

Template type		Reference amount	In general, template quantity
Genomic DNA	DNA from eukaryotic organisms	5-250ng	100ng
	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.

2.2. When using a rough sample template, please refer to the following data for the amount of addition (PCR reaction solution is 50μL).

Template type	In general, template quantity	Remarks
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 amount of vaccination needles	
Yeast	Pick a small amount of vaccination needles	
Filamentous bacteria	Pick a small amount of vaccination needles	
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

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

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

Note1)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)Loop count: When the number of copies of the target fragment is small, it is recommended to increase the loop count to 35-45 cycles.