Tribioscience

Pfu PCR MasterMix (2x) Catalog# TBS4012

Catalog	Unit
TBS4012	200 RXNs

DESCRIPTION

Pfu PCR MasterMix is an optimized 2 x premix reagent composed of Super Pfu DNA Polymerase, Mg2+, dNTPs, and reaction buffer. Super Pfu DNA polymerase has 3 ′ →5 ′ exonuclease activity, and its fidelity is about 100 times higher than Taq DNA polymerase, making it an ideal choice for cloning. This enzyme achieves high-speed PCR with an extension rate of 10 sec/kb while maintaining high fidelity through the introduction of extension enhancement technology. The amplification length can reach 20 kb. In addition, this product can amplify long fragments, GC rich regions, and excess templates that are difficult to amplify with high success rate. The PCR product obtained by amplification does not contain the "A" base at the 3 'end and can be directly cloned into a blunt ended vector.

APPLICATIONS

This kit is used for PCR amplification.

KEY FEATURES

- High-fidelity: The fidelity is about 100 times higher than Taq DNA polymerase. Long target fragments can also be amplified rapidly and with high fidelity.
- High-speed: When amplifying target fragments below 1kb, the extension time can be set to 10 seconds. When amplifying the target fragment of 1-10 kb, the extension time can be set to 20-30 sec/kb.
- Simple and convenient: This reagent contains all PCR components except primers and templates, which facilitates operation and improves the reproducibility of the results.

KIT CONTENTS

Component	Size
2x Pfu PCR MasterMix	1 mL
PCR Water	1 mL

STORAGE CONDITIONS

Store all components at -20°C in a non-frost-free freezer.
Shelf life is 12 months after receipt.
The kit is shipped on ice.

PROTOCOL

1. Prepare the following reaction mixture in PCR tube on ice

Components	Reaction Volume:20 µl
Total DNA	Variable
2x Pfu PCR MasterMix	10 µl
Forward Primer (10 µM)	0.5-1 µl
Reverse Primer (10 µM)	0.5-1 µl
PCR H_2O	Up to 20 µl

After adding the components, please mix them evenly and quickly transfer them to the PCR machine.

2. Set up PCR reaction program as below:

Step	Temperature	Duration	Cycle(s)
Pre-Denaturation	98°C	30 secs	1
Denaturation	98°C	5-10 secs	25-35
Annealing	Based on primer Tm	10-30 secs	
Extension	72°C	20-30 Sec/kb	
Final Extension	72°C	5 min	1

Note:

Pre denaturation: For most purified templates, 98℃ for 30 seconds is sufficient; For complex templates, the pre-denaturation time can be extended by no more than 3 minutes.

Annealing: In general experiments, the annealing temperature is 3-5℃ lower than the primer Tm. When non-specific reactions occur, the annealing temperature should be appropriately increased. If necessary, a temperature gradient can be established to find the optimal temperature for primer template binding. For high Tm primers, a two-step cycle can be used to combine annealing and extension into one step.

Extension: For complex genomic DNA templates, the extension time is usually 20–30 sec/kb. For simple templates (plasmids, E. coli, etc.), extension at 72°C for 5 minutes is usually sufficient.

RELATED PRODUCTS

One-step Sybr qRT-PCR (TBS4007)
2xSybr Green qPCR Mix (TBS4001)
2xTaqman Probe qPCR Mix (TBS4002)
2x Taqprobe RT-qPCR Mix (TBS4008)
RNA isolation Kit (TBS6001)
Ribospine vRD II Kit (Viral RNA isolation from cell-free fluid, plasma, Serum, Urine) (322-150)
Exgene Viral DNA/RNA Isolation Kit (128-150)

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