



Pfu DNA Polymerase

Product Number: PC0301

Shipping and Storage

-20°C

Components

Component	PC0301	PC0301
	100U	500U
Pfu DNA Polymerase,2U/μL	50μL	250μL
2×Pfu DNA Polymerase Buffer	2×1.25mL	7×1.8mL
dNTP Mix,10mM each	150μL	750μL

Description

Pfu DNA Polymerase is a fast and high-fidelity DNA Polymerase with high amplification efficiency. The Polymerase possesses 5'-3' DNA Polymerase activity and 3'-5' exonuclease activity. The enzyme was modified by other high-fidelity enzymes with strong amplification ability, fast amplification speed and high fidelity, which overcame the defects of ordinary Pfu enzyme such as poor amplification ability, low yield and slow amplification speed, and greatly shortened the reaction time. This product can be used for long fragment amplification and the expansion of other complex templates. The 3' end of the amplified PCR product does not contain "A" base, and can be directly cloned in the flat terminal vector. If T/A cloning is needed, "A" should be added to the end of the PCR product for cloning. This product is suitable for gene cloning, gene point mutation, SNP amplification experiments.

Unit definition

Absolute ethanol, 10mM PBS (PH7.4)

Quality Control

After multiple column purification, the purity was more than 98% by SDS-PAGE. No exogenous nuclease activity was detected. After one month at room temperature, there was no obvious change of activity.

Protocol

The following examples are the conventional PCR reaction system and reaction conditions, which should be improved and optimized according to the different template, primer structure and target fragment size in practice.

1. PCR Reaction System

All operations should be carried out on ice. After thawing, mix the components thoroughly. After use, please put them back to -20°C in time.

Reagent	50μL Reaction System	Final Conc.
2×Pfu DNA Polymerase Buffer	25μL	1×
dNTP Mix,10mM each	1.5-2.5μL	300-500μM each
Forward Primer,10μM	2μL	0.4μM
Reverse Primer,10μM	2μL	0.4μM
Template DNA (Appropriate)	Appropriate	<500ng/50μL
Pfu DNA Polymerase	0.5-0.75μL	1-1.5 U/50μL
ddH ₂ O	up to 50μL	

2. PCR Reaction Program

Step	Temperature	Time	Cycles
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Initialization	98°C	30sec-3min	1
Denaturation	98°C	10-30sec	} 25-35
Annealing	According to Tm	15-30sec	
Extension	72°C	3-5kb /min	
Final Extension	72°C	5min	1

Note: 1) The three-step amplification method is preferred. The three-step method cannot amplify the target product or primer with Tm value greater than 68°C, so please try the two-step method.

2) Denaturation: the pre-denaturation of simple templates is 98°C, 30s to 1min, and the pre-denaturation time can be extended to 3min for complex templates.

3) Annealing: in general experiments, the annealing temperature is 3-5°C lower than the Tm value of the primer. If the ideal amplification efficiency cannot be obtained, the annealing temperature should be changed gradient for optimization; When non - specific reaction occurs, the annealing temperature should be increased appropriately.

4) Extension: The extension time should be set according to the length of the amplified fragment and the complexity of the template. The amplification efficiency of this product is 3-5 KB /min, and 2-4 KB /min is recommended for long fragments and templates with high complexity.

5) Cycle number: the cycle number can be set according to the downstream application of the amplification product. If the cycle number is too small, the expansion increment is insufficient, and the cycle number is too large, the mismatch probability will increase. Therefore, the cycle number should be reduced as far as possible on the premise of ensuring the yield of the product.as possible yet ensuring the yield of the product.