

Catalog	Unit
TBP0145-500	500U
TBP0145-1000	1000U

### Description

Pfu DNA Polymerase is essential for accurate and reliable PCR applications. It possesses both 5'–3' polymerase activity and 3'–5' exonuclease (proofreading) activity, ensuring high precision during DNA synthesis. It is particularly suitable for amplifying long or complex DNA fragments and is ideal for applications such as gene cloning, site-directed mutagenesis, and SNP analysis.

### Product Details

**Unit definition:** Absolute ethanol, 10mM PBS (PH7.4)

**Purity:** 98% by SDS-PAGE

**Storage:** -20°C

### Components

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

### 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 a time.

2.

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	1-1.5U/50μL
ddH <sub>2</sub> O	Up to 50μL	

3.

PCR Reaction Program			
Step	Temperature	Time	Cycles
Initialization	98°C	30sec-3min	1
Denaturation	98°C	10-30sec	25-35
Annealing	According to T <sub>m</sub>	15-30sec	25-35
Extension	72°C	3-5kb /min	25-35
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 T<sub>m</sub> 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 T<sub>m</sub> 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.

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