Tinzyme Co., Limited

Email: sales@tinzyme.com Website: www.tinzyme.com
Tel: +86-755-86134126 WhatsApp/Facebook/Twitter: +86-189-22896756

Kfu DNA Polymerase

Product Number: PC312

Shipping and Storage

-20°C.

Components

Component	PC312 100U	PC312 500U
Kfu DNA Polymerase,2U/μL	50μL	250μL
2× Kfu Buffer	2×1.25mL	7×1.8mL
dNTP Mix，10mM each	150μL	750μL

Description

Kfu DNA Polymerase is a high fidelity DNA polymerase with fast and high amplification efficiency, which has 5'-3' DNA polymerase activity and 3'-5' exonuclease activity.This enzyme was modified by other high-fidelity enzymes, adding unique elongation factors and specific promoter factors, greatly improving amplification ability, overcoming the shortcomings of poor amplification ability, low yield, and slow amplification speed of ordinary Pfu enzymes, and shortening reaction time.This product can be used for amplification of ordinary fragments, long fragments, and various other complex templates. The 3 'end of the PCR product obtained from amplification does not carry an "A" base. If T/A cloning is required, "A" needs to be added to the end of the PCR product before cloning.This product is suitable for gene cloning, second-generation library building amplification, gene directed mutation, SNP and other amplification experiments.

Unit definition

The amount of enzyme required to add 10 nmoL deoxyribonucleotides to acidic insoluble substances within 30 minutes at 74 ℃ is defined as 1 active unit (U).

Quality Control

After multiple column purification, the purity was detected by SDS-PAGE to be greater than 98%; No exogenous nuclease activity was detected; Store at room temperature for one month without significant changes in activity.

Protocol

The following examples are the conventional PCR reaction system and reaction conditions. In practical operation, corresponding improvements and optimizations should be made based on different templates, primer structures, and target fragment sizes.

1. PCR reaction system

All operations should be carried out on ice. After each group is decomposed and frozen, please mix thoroughly. After use, please put it back at -20℃ for storage in a timely manner.

Reagent	50 μL reaction system	Final Conc.
2×Kfu Buffer	25μL	1×
dNTP Mix，10 mM each	1.5-2.5μL	300-500μM each
Forward Primer, 10μM	2μL	0.4μM
Reverse Primer, 10μM	2μL	0.4μM
Template DNA appropriate amount	Appropriate amount	<500 ng/50μL
Kfu DNA Polymerase	0.5-0.75μL	1-1.5 U/50μL

ddH_2O	up to 50μL

2. PCR reaction conditions:

Step	Temperature	Time	
Pre denaturation	98°C	30s-3mins	
Denaturation	98°C	10-30s	25-35cycles
Annealing	Based on primer Tm	15-30s	
Extend	72°C	4-6kb/min	
Final extension	72°C	5mins	

Note:1)Priority should be given to using the three-step amplification method. If the three-step method cannot amplify the target product or the Tm value of the primer is greater than 68°C, please try the two-step method.

2)Denaturation: Pre denaturation of simple templates at 98°C for 30 seconds to 1 minute. For complex templates, the pre denaturation time can be extended to 3 minutes.

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 achieved, the annealing temperature should be gradient changed for optimization; When non-specific reactions occur, increase the annealing temperature appropriately.

4)Extension: The extension time should be set based on the length of the amplified fragment and the complexity of the template. The amplification efficiency of this product is 4-6 kb/min, and 2-4 kb/min is recommended for long fragments and high complexity templates.

5)Number of cycles: The number of cycles can be set based on the downstream application of the amplified product. If the number of cycles is too small, the amplification amount is insufficient, and the number of cycles is too many, the probability of mismatch will increase. Therefore, while ensuring product yield, the number of cycles should be minimized as much as possible.