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Fast Pfu DNA Polymerase

Product Number:PC0302

Storage Conditions

-20°C

Shelf Life

3 years

Concentration

5U/μl

Description

The enzyme is a heat-resistant DNA polymerase with high fidelity, and its fidelity is 8~10 times that of Taq DNA polymerase. The enzyme has not only 5'-3'DNA polymerase activity, but also 3'-5'exonuclease proofreading activity. It can timely remove the base mismatched nucleotides in the DNA synthesis chain, greatly increasing the correct rate of base pairing and ensuring the high fidelity of DNA synthesis. The enzyme has no 5'-3'exonuclease activity. The synthesis speed is 15 seconds 1KB, and the effective amplification length is more than 8KB.

Source

E. Coli recombinant strain, containing the gene cloned from Pyrococcus furiosus and modified.

Unit Definition of activity

Take calf thymus DNA as template, react at 72°C for 30min, and the amount of enzyme that can catalyze the polymerization of 10nmol dNTP into polynucleotide fragments is defined as 1 active unit (U)

Storage buffer

[PH8.0] 20 mM Tris-HCl, 100 mM KCl, 0.1 mM EDTA, 0.1 % NP-40 (v/v), 0.1 % Tween-20(v/v), 1 mM DTT, 50 % glycerol(v/v).

Reaction buffer

[10×Pfu Buffer (Mg^{2+})] 200 mM Tris-HCl (PH 8.8), 100 mM KCl, 160 mM (NH4)2SO_4, 1 % Triton X-100, 1 mg/ml BSA, 20 mM $MgSO_4$

Protocol

User prepared reagent: dNTP mixture; Water (without nuclease); Primers (upstream and downstream); Template

1. Establishment of PCR reaction system

After all the solutions are completely melted, shake gently and fully;

Add into thin-walled PCR tube and operate on ice; fifty μ L the reaction system is as follows:

Component	Addition volume (μl)	Final concentration
10× Buffer with Mg^{2+}	5	1×
dNTP Mix,10mM	1	0.2 mM
Upstream primer	X	0.1-1.0μM
Downstream primer	Y	0.1-1.0μM
pfu (5U/μl)	0.5	2.5U
DNA Template	Z	<0.5μg/50μl

Add water to	50μl	-

Note: PFU enzyme should be added after dNTPs is added, otherwise the fidelity activity of PFU enzyme may degrade the primer, resulting in the production of non-specific bands and the decline of product yield.

2. PCR reaction cycle setting

Program	Temperature	Time	cycles
Pre denaturation	95°C	2 min	1
Denaturation	95°C	2 min	25-35
Annealing	42-65°C	0.5-1 min	
Extend	72°C	10-15s /kb	
Fina extension	72°C	5 min	1

3. Result test

Take 5 after reaction μ L reaction products were detected by agarose gel electrophoresis.