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RealPfu™ DNA Polymerase

Most Accurate Amplification

Store at -20°C

Cat. No. RT004
500 units, with dNTP
RealPfu™ DNA Polymerase (5 U/µl): 100 µl
10 X Reaction Buffer (with 20 mM Mg^{2+}) : 2 ml
10 mM dNTPs Mix: 200 µl

Cat. No. RT044
500 units, without dNTP
RealPfu™ DNA Polymerase (5 U/µl): 100 µl
10 X Reaction Buffer (with 20 mM Mg^{2+}) : 2 ml

Recombinant	✓
5′ to 3′ Exonuclease	✓
3′ to 5′ Exonuclease	✓
Endonuclease Free	✓

Description

RealPfu™ DNA Polymerase is purified from Pyrococcus furiosus. The thermostable enzyme catalyzes the incorporation of nucleotides into duplex DNA in the 5'--3' direction in the presence of Mg2+ at 70-80°C. RealPfu™ DNA Polymerase exhibits both 3'--5' exonuclease (proofreading) activity and 5'--3' exonuclease activity, which leads to the lowest error rate and the most reliable high-fidelity PCR.

Unit Definition

One unit of enzyme catalyzes the incorporation of 10 nmol of dNTP into acid-insoluble form in 30 minutes at 74°C.

Error Rate

The error rate of RealPfu™ DNA Polymerase is $1x10^{-6}$ errors per nucleotide per cycle.

Storage Buffer

50 mM Tris-HCl (pH 9.0), 100 mM NaCl, 0.1 mM EDTA, 1% Triton X-100, 5 mM DTT, 50% Glycerol, Stabilizers

10X Reaction Buffer

100 mM KCl, 20 mM $MgSO_4 \cdot 7H_2O$, 200 mM Tris-HCl (pH 8.8), 1% Triton X-100, 100 mM $(NH_4)_2SO_4$, 1 mg/ml BSA.

Quality Control

Nuclease activity was not detected after incubation of 1 µg lambda/HindIII DNA with 5 units of RealPfu™ DNA Polymerase in 50 µl reaction volume reaction buffer for 18 hours at 37°C.

General Reaction Conditions

The optimal conditions for the concentration of RealPfu™ DNA Polymerase, primers and template DNA will depend on the system being utilized. It may be necessary to determine the optimal conditions for each individual component.

1. Add the following components to a sterile microtube on ice :

Components	Volume	Final Concentration
10X Reaction Buffer	5 µl	1X
10 mM dNTP Mix	0.5 µl	0.1 µm
Primer Mix (10 µM each)	1 µl	0.2 µm
Template DNA	0.5~10 µl	n/a
RealPfu™ DNA Polymerase(5U/µl)	0.5 µl	2.5 units
ddH_2O	add to 50 µl	n/a

2. Suggested Reaction Parameters for RealPfu™ DNA Polymerase :

Segment	Number of Cycles	Temperature	Duration
1	1	94°C	5 minutes
2	25~35	94°C (Denature)	30 seconds
		Primer Tm-5°C (Anneal)	30 seconds
		68°C (Extend)	2 minutes/Kb
3	1	68°C	7 minutes
		4°C	∞

3. Analyze the amplification products by agarose gel electrophoresis and visualize by ethidium bromide staining.

Note: For research use only. Not for use in diagnostic or therapeutic procedures.

RBC
It's the Joy of Discovery

Real Biotech Corporation