Kyratec.

Product Datasheet

X-*Pfu* DNA Polymerase

Cat No.

XT64002	250 Units
XT64005	500 Units

Features

- Ultra pure recombinant protein.
- Recommended for use in high-fidelity amplification, amplification of GC-rich sequences or problematic secondary structures, primer extension reactions at elevated temperatures and cloning of blunt-ended amplification products.

Description

X-*Pfu* DNA Polymerase is an extremely thermostable proofreading DNA polymerase, suitable for applications requiring high temperature synthesis of DNA. DNA Polymerase catalyzes the polymerization of nucleotides into duplex DNA in the 5' to 3' direction in the presence of Mg^{2+}. It exhibits the 3' to 5' proofreading activity, resulting in over 10-fold higher fidelity than possible with Taq DNA Polymerases.

Product Specifications

Concentration: 5U/ul

Supplied with

	250 Unit	500 Unit
X-*Pfu* DNA Polymerase	50 uL	100 uL
10x Buffer A	1.2 mL	2 x 1.2 mL
50mM $MgCl_2$	1 mL	1 mL
10mM dNTP mix	1 mL	1 mL

Reation Buffer

10X Buffer A (without $MgCl_2$):
500mM KCl, 100mM Tris-HCl (pH 9.1 at 20°C) and 0.1% Triton™ X-100. The buffer is optimized for use with 0.1 - 0.2mM of each dNTP.

Storage Buffer

20mM Tris-HCl (pH 8.0 at 22°C), 100 mM KCl, 0.5% Tween™ 20, 0.5% Nonidet-P40, 0.1mM EDTA, 1mM DTT and 50% glycerol.

Storage Conditions

X-*Pfu* DNA Polymerase can be stored for 12 month at -20°C.

Shipping Conditions

On Dry Ice or Blue Ice.

Quality Control

All preparations are assayed for contaminating endonuclease, exonuclease, and non-specific DNase activities. Functionally tested in DNA amplification.

Unit Definition

1u is defined as amount of enzyme that required to catalyze the incorporation of 10nmoles of dNTP into acid-insoluble material in 30 minutes at 74°C.

PCR Reaction Conditions (for a 50uL reaction)

10x Buffer A	5 uL
X-*Pfu* DNA Polymerase (5 units/uL)	2.0 - 2.5 uL
50mM $MgCl_2$	1.0 - 2.0 uL
10mM dNTP mix	1.0 uL
Template	〈 500 ng
Primers	0.2 - 1.0 uM
Water (ddH_2O)	up to 50 uL

PCR Condition

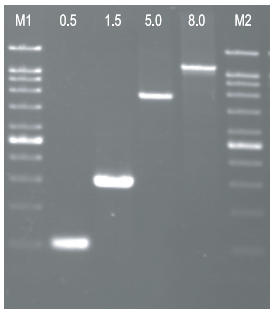
PreDenaturation	94°C, 2 min
Denaturation	94°C, 30 sec
Annealing	50 - 68 °C, 30 sec
Extension/1kb	72°C, 30 sec
Cycles	35 cycles
Final Extension	72°C, 7 min

Notes:

Denaturation condition varies depending on an used thermal cycler and tube. It is recommended for 10 - 30 sec. at 94°C.

The suggested final concentration of Mg^{2+} in the reaction is likely to be 2 - 4 mM, but some optimization may necessary to achieve the best possible results.

This data is intended for use as a guide only; conditions will vary from reaction to reaction and may need optimization.



Amplification Using X-Pfu DNA Polymerase

Lane M1 : 1kb DNA Ladder
Lane 0.5 and 1.5kb : 0.5kb ampification product generated using 0.2mM dNTPs and 2.0u X-Pfu DNA Polymerase.
Lane 5kb and 8kb : 5kb and 8kb amplification products generated using 0.25mM dNTPs, 2.5u X-Pfu DNA Polymerase and 3% of formamide.
Lane M2 : Lambda / Hind III Marker
0.7% TAE agarose gel