

Lot No.

Contents

IP- <i>Pfu</i> DNA Polymerase (2.5 U/μl)	250 U X 2
10X IP- <i>Pfu</i> Buffer	1 ml X 2
5X Tuning Buffer	1 ml
dNTPs (Each 2.5 mM)	0.5 ml X 2
Store at -20°C	

Description

Labopass™ IP-*Pfu* DNA Polymerase is a thermostable DNA polymerase cloned from *Pyrococcus furiosus* and a recombinant form expressed in *E.coli*. This archaeal polymerase possesses 3'-5' exonuclease proofreading activity together with 5'-3' polymerase activity, which allows high fidelity DNA amplification. *Pfu* polymerase remains its polymerase activity during extended exposure at 98°C unlike Taq polymerase. Therefore, this enzyme can be used to amplify difficult templates (e.g. high GC content or stable secondary-structure).

Applications

- High fidelity PCR
- Preparation of PCR products for cloning
- Site-directed mutagenesis
- Blunting of DNA ends

Unit definition

One unit is defined as the amount of enzyme that incorporates 10 nmoles of dNTPs into acid-insoluble form in 30 min at 72°C.

Storage buffer

50 mM Tris-HCl (pH8.2), 50 mM KCl, 0.1 mM EDTA, 1 mM DTT, 0.1% Tween-20, 0.1 % NP-40, 50 % glycerol

Purity

Nicking, endonuclease and exonuclease activity were not detected after the incubation of 0.5 μg of supercoiled pUC19, λDNA or HindIII digested λDNA with 10 units of this enzyme for 4 hour at 37°C or 72°C.

10X IP-*Pfu* buffer (with MgCl₂)

Labopass™ IP-*Pfu* DNA Polymerase is supplied with an optimized reaction buffer for improved PCR yield.

5X Tuning buffer

Tuning buffer can improve PCR efficiency in reaction using problematic template DNA containing high GC contents or stable secondary structure. Thus, it is advantageous to amplify complicated long target sequences.

Standard reaction (50 μl)

Components	Volumes (μl)
10X IP- <i>Pfu</i> Buffer	5 μl
dNTPs (Each 2.5 mM)	4 μl
5X Tuning buffer	10 μl (optional)
Forward Primer	10 ~ 50 pmoles
Reverse Primer	10 ~ 50 pmoles
DNA Template	variable *
LaboPass™ IP- <i>Pfu</i> DNA Polymerase	0.5 ~ 2 units
Distilled water	up to 50 μl

* Amount of DNA template

- | | |
|---|-----------|
| - Eukaryotic genomic DNA | 10-200 ng |
| - Prokaryotic genomic DNA | 1-50 ng |
| - Purified homogeneous DNA
(e.g. plasmid, lambda DNA. etc) | <5 ng |