Pro *Pfu* polymerase Kit

Cat# no.: A9005

Store at -20 C

For Research use Only

1.1 Ordering Information

Kit components	A9005-10	A9005-25	A9005-50	A9005-100
Pro fidelity *Pfu* DNA Polymerase	10µL	25µL	50µL	100µL
10X PCR Reaction buffer	20µL	50µL	100µL	200µL
dNTPs (2.5mM)	20µL	50µL	100µL	200µL
Nuclease-free water	500µL	1mL	1.5ml	1mL x 2

1.2 Introduction

Polymerase Chain Reaction, most commonly known as PCR, is a technique based on the ability of DNA polymerase to synthesize complementary DNA strand to the given template strand. It allows amplification of very small sample or a short segment of DNA into millions and billions of copies rapidly. It is the most common and indispensable technique in a research and diagnostic laboratory.

1.3 Description

The ProEnz Pro Pfu polymerase kit consists of 10X Pfu PCR buffer and Pro fidelity Pfu polymerase enzyme for amplification of the DNA fragment to billions of copies. The Pro Pfu Polymerase kit is recommended for use in PCR reactions that require high-fidelity synthesis.

Pfu DNA polymerase is a thermostable enzyme of approximately 90 kDa, purified from the expression product of *E. coli* strain carrying the *pol* gene from Pyrococcus furiosus. The enzyme catalyzes the polymerization of nucleotides into duplex DNA in 5' to 3' direction, in the presence of Mg2+, creating PCR fragments with blunt ends. It possesses 3' to 5' exonuclease (proof-reading) activity and is recommended for use in reactions that require high-fidelity synthesis.

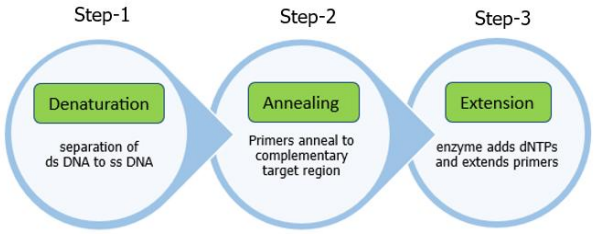
1.4 Salient features

- Possesses proof-reading 3'-5' exonuclease activity.
- High-fidelity synthesis.
- Generates blunt-end PCR products.

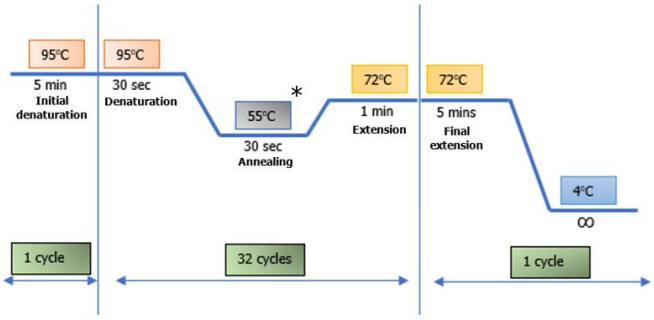
1.5 Principle of the technique

In PCR, a short segment of DNA is amplified using primer mediated enzymes. The DNA polymerase adds a nucleotide to the pre-existing 3'-OH group of the primers and synthesizes new strand complementary to the template strand, extending the desired target region.

1.6 PCR steps



1.7 PCR conditions



* Annealing temperature can vary with primers.

1.8 Applications

1. For DNA amplification by Polymerase Chain Reaction (PCR).
2. For DNA cloning, DNA fragments, stitching, SNP,

gene synthesis that demands high fidelity.

1.9 Storage & handling

Store @-20 C

1.10 Protocol

1. Place all the components on ice for thawing 20-30 mins prior.
2. After ensuring that all the components are thawed completely, gently vortex the components and give a brief spin and place the vials on ice.
3. Place 0.2mL PCR tubes, for the required number of reactions, on ice and add the components for 20µl reaction volume as given in Table-1.

Table-1:

Components	Concentration	Vol. for 20 µL
Pro *Pfu* DNA polymerase	2.5U	0.5 µL
10x PCR Buffer	1X	2 µL
Forward Primer	0.1-0.5 µM	0.5-1 µL
Reverse Primer	0.1-0.5 µM	0.5-1 µL
dNTP (2.5 mM each)	0.2mM-0.4mM	2 µL
Template	10pg-500ng	Variable
Nuclease free water	-	Make up to 20 µL

4. It is recommended to add the enzyme as the last component to the tube.
5. After ensuring that all components have been added to the tube, spin the tubes in a microcentrifuge and ensure no bubbles are visible in the tubes.
6. Place these vials in a thermal cycler and perform PCR with conditions as given in the section- 1.7
7. Start the run and note the end time.
8. In the meantime, prepare an agarose gel of desired concentration. After solidification, submerge the gel in 1X TAE/TBE buffer.
9. After the PCR run, take out the tubes and add DNA loading dye to the tubes and load the samples on the gel and run till the dye front can be seen 3/4th the gel.
10. Observe the gel under UV light for desired bands.

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1.11 Unit definition

One unit is the amount of enzyme that will incorporate 10 nmol of dNTP into acid- insoluble products in 30 minutes at 72°C with GAPDH mice DNA as template primer.

1.12 Must follow

Store enzyme and buffer at -20°C after resuspension.
If turbidity/precipitation is observed in buffer and enzyme, discard the vials immediately.

1.13 10X PCR buffer provided with Mg^{+2} composition

200mM Tris-HCl (pH 8.8), 100mM $(NH_4)_2SO_4$, 100mM KCl, 20mM $MgSO_4$, 1% Triton X-100.

1.14 Quality control tests

Compositions of the 10X reaction buffers have been optimized to assure quality performance of the enzyme under a variety of conditions. Good performance of DNA amplification by PCR was confirmed by using λDNA as the template (amplified fragment: 8kb).

1.15 Materials required but not supplied

1. Template
2. 0.2ml PCR tubes
3. Forward and reverse primers
4. Pipette tips
5. Agarose
6. 1X TAE/TBE buffer
7. DNA loading dye
8. DNA ladder

1.16 Limitations

1. The specificity of the generated PCR product may be altered by non-specific binding of the primers to other similar sequences on the template DNA.
2. In order to design primers to generate a PCR product, some prior sequence information is usually necessary.
3. DNA polymerases are prone to error, which potentially causes mutations in PCR products.
4. PCR is highly sensitive to contamination.

1.17 Optimal results can be obtained by:

1. Clean the working bench with 70% ethanol or isopropyl alcohol before placing anything on the bench.
2. Wear a fresh pair of protective nitrile powder-free gloves before setting up the reaction.
3. Let all the components thaw on ice completely prior to vortex them.
4. Avoid repeated freeze-thaw cycles.
5. To avoid repeated freeze-thaw cycles, aliquot enzyme and buffer with volume required for single use.
6. Use properly calibrated pipettes.

1.18 Troubleshooting

The first step is to confirm that all the reagents are added to the vial and the reagents are not contaminated.

Problem: No or low PCR yield

Possible cause	Solution
Low or high reagent concentration	Adjusting final concentration. See Table-1 in section-1.10 for appropriate concentrations.
Cycling conditions	Tweak the cycling conditions as per template & primer concentration.
Pipetting errors	Make sure to use calibrated pipettes

Note: Use pre-mixed **ProEnz 2X Pfu master mix Cat# PE2402** for easy assembly and reduce pipetting errors. Just add primers, template and water to the recommended volume of the master-mix.

Problem: Primer-dimer formation

Possible cause	Solution
Poor primer specificity	Check the target specificity of the primers using online primer tools. The annealing temperature is 5°C less than the Tm of the primer pair.
Primer concentration	Primer concentration can range anywhere between 0.1µM -1µM.

Problem: False positives

Possible cause	Solution
Contamination of previous results	Make sure to dedicate a different space for pre-PCR set-up and post-PCR run.
Non-specific binding	Set-up the reaction on ice.

Explanation of symbols

REF -Catalogue number

- Expiry

LOT - Lot number/Batch number

-25°C max
-15°C min
- Storage limitations

-Manufacturing: Plot No. 147/D, Phase II, Cherlapally IDA, Telangana, Hyderabad - 500051, India.

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