

Pfu DNA polymerase

Description:

Pfu DNA Polymerase is a thermostable enzyme with a molecular weight of 90 kDa. It catalyzes the polymerization of nucleotides into duplex DNA in the 5'_3' direction, resulting in blunt-ended PCR products **without 3'-dA overhangs**.

Pfu DNA Polymerase exhibits 3'_5' exonuclease (proofreading) activity that enables the polymerase to correct the mis-incorporation of nucleotide, and lacks 5'_3' exonuclease activity. It is suitable for PCR and primer extension reaction that requires high fidelity when the PCR fragment is relatively **shorter than 3kb**.

The enzyme exhibits 3'>5' proofreading activity, resulting in over 10-fold higher PCR fidelity than possible with Taq DNA Polymerases.

Buffers and Reagents:

Storage Buffer: 20 mM Tris-HCl (pH 8.0), 100 mM KCl, 0.5 mM EDTA, 0.1 mM DTT, 0.5% Tween 20, 0.5 % Nonidet P-40, 50% Glycerol.

5X Reaction Buffer: Contains Tris-HCl (pH 9.0), PCR enhancers, KCl, (NH₄)₂SO₄

Contents:

| Components | C101141 | C101142 |
|--|---------------|--------------|
| Pfu DNA poly. 5 U/μl | 250 U | 500 U |
| MgCl₂ Solution 25 mM | 0.5 ml | 1 ml |
| 5X Buffer (MgCl₂ free) | 0.5 ml | 1 ml |

Kit storage: This kit should be stored at -20°C. Unnecessary repeated freeze/thawing should be avoided. Under these conditions reagents are stable for one year from the date of production.

General Reaction Protocol:

Please be sure to follow below

1. Thaw 10X reaction buffer , dNTP mixture.

| Component | Vol (μL) |
|---------------------------------------|-----------|
| 5X Reaction Buffer | 4 μL |
| MgCl₂ Solution 25mM | 2 μL |
| 40 mM dNTPs Mix (10 mM each) | 0.5 μL |
| Upstream Primer (10 pmol/μl) | 1 μL |
| Downstream Primer(10 pmol/μl) | 1 μL |
| Pfu DNA poly. (5 units/μL) | 0.25 μL |
| Template DNA | Variable |
| Sterilized D.W. | Variable |
| Total Volume | 20 μL |

2. Mix the master mix thoroughly and dispense appropriate volumes into PCR tubes or plates.
3. Add templates DNA to the individual PCR tubes or wells containing the master mix.
5. Program the PCR machine according to the program outlined.

6. Place the PCR tubes or PCR plates in the terminal cyclor and start the cycling program.

7. Separate the PCR products by agarose gel electrophoresis and visualize with EtBr or any other means.

* Longer extension time makes nonspecific bands

| Cycle | Time | Temp °C |
|---------------|--------|---------|
| 1 | 3 Min | 94 |
| | 30 Sec | 94 |
| 25 ~35 | 30 Sec | 50~60 |
| | 30 Sec | 72* |
| 1 | 3 Min | 72 |

*Extension rate for this enzyme is near 1000 bp/min.

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