# *pfu* DNA Polymerase

**Size**:

| Cat.# | Size |
|---|---|
| BG10301S | 100 U |
| BG10301M | 500 U |
| BG10301L | 2500 U |

**Concentration:** 2.5 units/ μL

**Source:** *E.coli* cells with a cloned *pfu* DNA polymerase from Pyrococcus furiosus strain

**Storge temperature:** -20℃ .

**Exp.:** 12 months(-15℃ ~ -25℃ )

**Applications**

1. High fidelity PCR
2. Blunt-ended PCR cloning

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**Description:**

*pfu* DNA Polymerase catalyzes the polymerization of nucleotides into duplex DNA in the 5'→3' direction in the presence of $Mg^{2+}$ at 75°C. It exhibits 3'→5' exonuclease activity that enables to correct error extension, but no 5' → 3' exonuclease, recommended for use in PCR that require high fidelity. PCR fragments generated by *pfu* DNA Polymerase are blunt-ended.

**Reagent supplied:**

| Cat#. | Size | Reagent | No. |
|---|---|---|---|
| BG10301S | 100 U | 10 × *pfu* DNA Polymerase Reaction Buffer | 1.5 mL |
| BG10301M | 500 U | 10 × *pfu* DNA Polymerase Reaction Buffer | 2 × 1.5 mL |
| BG10301L | 2500 U | 10 × *pfu* DNA Polymerase Reaction Buffer | 10 ×1.5 mL |

**Quality Control:**

1. Amplification ability: 25 cycles of PCR amplification of 5 ng Lambda DNA in the expected 5 kb product.
2. Nuclease Activity: No contaminating endonuclease or exonuclease activity have been detected
3. Protein Purity (SDS-PAGE): pfu DNA Polymerase is >99% pure judged by SDS-PAGE.
4. qPCR for DNA Contamination(*E.coli* Genome): A minimum of 5 µL of *pfu* DNA Polymerase is screened for the presence of *E.coli* genomic DNA using qPCR with Taqman Probe specific for *E.coli* 16S rRNA.The measured level of *E.coli* genomic DNA contamination is less than 10 copies of *E.coli* genome.

**Definition of activity unit:**

One unit is defined as the amount of enzyme required to catalyze the incorporation of 15nM of dNTPs into acid insoluble material in 30 minutes at 75°C.

**Storage buffer**:

1mM DTT, 0.1mM EDTA, 100mM KCl, 20mM Tris-HCl (pH 8.2 @ 25°C), 0.1%(v/v) NP-40, 0.1%(v/v) Tween 20, 50%(v/v) glycerol.

**Protocol**:

To prepare several parallel reactions and to minimize the possibility of pipetting errors, prepare a PCR master mix by mixing Nuclease-Free water, buffer, dNTPs and primers. Prepare sufficient master mix for the number of reactions plus one extra. Aliquot the master mix into individual PCR tubes and then add template DNA. It is critical to withhold *pfu* DNA Polymerase at last, otherwise, the proofreading activity of the polymerase may degrade the primers.

1. Gently vortex and briefly centrifuge all solutions after thawing.
2. Place a thin-walled PCR tube on ice and add the following component for each 25 µL or 50 µL reaction:

| Component | 25µl reaction | 50 µl reaction | Final concentration |
|---|---|---|---|
| 10 × pfu DNA Polymerase Reaction Buffer | 2.5 µl | 5 µl | 1X |
| dNTP Mix (10 mM each) | 0.5 µl | 1 µl | 200 µM each |
| 10 µM forward primer | 0.5 µl | 1 µl | 200 nM |
| 10 µM reverse primer | 0.5 µl | 1 µl | 200 nM |
| Template DNA | variable | variable | 0.1-100 ng |
| pfu DNA polymerase | 0.25 µl | 0.5 µl | 1.25U/50 µL |
| Nuclease free water | to 25 µl | to 50 µl | – |

3. Gently vortex the samples and spin down.Place the reaction in a thermal cycler that has been preheated to 95°C for 1-2min, but longer than 2min is unnecessary.
4. Perform PCR using recommended thermal cycling conditions:

| Step | Temperature | Time | Number of cycles |
|---|---|---|---|
| Initial Denaturation | 95°C | 1-2 min | 1 |
| Denaturation | 95°C | 30-60 s | 25-35 |
| Annealing | 42-65°C | 30 s | |
| Extension | 72-74°C | 2-4 min/kb | |
| Final Extension | 72-74°C | ∞ | 1 |

**General guidelines:**

1. Enzyme Concentration:

We recommend that 1.25 units of *pfu* DNA Polymerase be used per 50 μl amplification reaction. The inclusion of more enzyme will increase the likelihood of primer degradation due to the intrinsic 3' → 5' exonuclease (proofreading) activity. It is essential to withhold *pfu* DNA Polymerase from the reaction after the addition of the dNTP mix and to assemble components on ice.

2. Template:

High quality, purified DNA templates greatly enhances the success of PCR reaction. Recommended amounts of DNA template for a 50 µl reaction are as follows:

| DNA | Amount |
|---|---|
| Genomic DNA | 1 ng-1 µg |
| Plasmid or Viral DNA | 1 pg-1 µg |

3. Primers:
Oligonucleotide primers are generally 20-40 nucleotides in length and ideally have a GC content of 40-60%. Computer programs such as Oligo 7 can be used to design or analyze primers. The final concentration of each primer in a PCR reaction may be 0.05-1 μM, typically 0.1-0.5 μM.
4. Annealing:
The annealing step is typically 30 s. Annealing temperature is based on the Tm of the primer pair and is typically 42~65°C. Annealing temperatures can be optimized by doing a temperature gradient PCR.
5. Extension:
The recommended extension temperature is 72-74°C. Extension times are generally 2 min/kb(minimum extension time of 1min/kb)

**Reference:**


1. Fiala G, Stetter K O. Pyrococcus furiosus sp. nov. represents a novel genus of marine heterotrophic archaebacteria growing optimally at 100°C[J]. *Archives of Microbiology*, 1986, 145(1) : 56-61.
2. Lundberg K S, Dan D S, Adams M W W, et al. High-fidelity amplification using a thermostable DNA polymerase isolated from Pyrococcus furiosus. Gene[J]. *Gene*, 1992, 108(1) :1-6.
3. Cline, J., Braman, J.C. and Hogrefe, H.H. (1996) PCR fidelity of Pfu DNA polymerase and other thermostable DNA polymerases. *Nucl. Acids Res.* 24, 3546–51.


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# *pfu* DNA 聚合酶使用说明书

【产品名称】*pfu* DNA 聚合酶

【产品规格】

| 货号 | 规格 |
|---|---|
| BG10301S | 100 U |
| BG10301M | 500 U |
| BG10301L | 2500 U |

【产品浓度】2.5 units/μL

【产品来源】重组表达强烈嗜热球菌 DNA 聚合酶基因的大肠杆菌

【储存条件】-20 ℃ 【保质期】24 个月（-15℃ ~ -25℃）

【应用范围】1. 需要有较高保真度的 PCR 反应 2. 反应产物需要平端片段的 PCR 反应

【产品说明】*pfu* DNA 聚合酶在镁离子存在的条件下，75℃时可以催化脱氧核苷酸沿着 5' 端→ 3' 端发生聚合反应，形成双链 DNA。同时它具有 3' 端→ 5' 端的核酸外切活性，因此具有校正功能，无 5' 端→ 3' 端核酸外切活性。推荐使用 *pfu* DNA 聚合酶进行有较高保真要求的 PCR 反应。另外需要注意的是 *pfu* DNA 聚合酶产生的 PCR 产物是平末端。

【随酶提供试剂】

| 货号 | 规格 | 试剂 | 数量 |
|---|---|---|---|
| BG10301S | 100 U | 10 × *pfu* DNA 聚合酶反应缓冲液 | 1.5 mL |
| BG10301M | 500 U | 10 × *pfu* DNA 聚合酶反应缓冲液 | 2 × 1.5 mL |
| BG10301L | 2500 U | 10 × *pfu* DNA 聚合酶反应缓冲液 | 10 × 1.5 mL |

【质量控制】扩增能力：5 ng 的 λ DNA 经过 25 个循环的扩增得到预期的 5 kb PCR 产物。2. 核酸酶活性检测：没有检测到外源的核酸内切酶和核酸外切酶活性。3. 蛋白纯度（SDS-PAGE）：SDS-PAGE 检测纯度 >99%。4. qPCR 检测 DNA 污染（大肠杆菌基因组）：通过 Taqman 探针 qPCR 法检测大肠杆菌 16S rRNA 位点，用至少 5 μL 的 *pfu* DNA 聚合酶来检测，结果显示大肠杆菌基因组 DNA 的污染水平小于 10 个大肠杆菌基因组。

【单位定义】1 单位的 *pfu* DNA 聚合酶为在 75℃，30min 的条件下聚合 10nM dNTP 所需要的酶量。

【贮存条件】1mM DTT, 0.1mM EDTA, 100mM KCl, 20mM Tris-HCl (pH 8.2 @ 25°C), 0.1%(v/v) NP-40, 0.1%(v/v) Tween 20, 50%(v/v) 甘油。

【操作指南】如果要同时进行几个平行反应，混合无核酸酶水，缓冲液，dNTPs，引物制备 PCR 混合液，为减少加样误差的影响，制备的反应混合液要在需要的反应数量上添加上一个额外反应，分装母液到单个的 PCR 管中，然后加入模板 DNA。最后再加入 pfu DNA 聚合酶，防止 pfu DNA 聚合酶降解引物。
1. 温和震荡后，快速离心解冻后的所有溶液。
2. 加入 25 μL 或者 50 μL 的组份（见下表）到放置在冰上的薄壁 PCR 管中。

| 成分 | 25 μL 反应 | 50 μL 反应 | 终浓度 |
|---|---|---|---|
| 10 × *pfu* DNA 反应缓冲液 | 2.5 μL | 5 μL | 1 × |
| dNTP 混合物（各 10 mM） | 0.5 μL | 1 μL | 各 200 μM |
| 10 μM 正向引物 | 0.5 μL | 1 μL | 200 nM |
| 10 μM 反向引物 | 0.5 μL | 1 μL | 200 nM |
| 模板 DNA | 不同量 | 不同量 | 0.1-500 ng |
| *pfu* DNA 聚合酶 | 0.25 μL | 0.5 μL | 1.25U/50 μL |
| 无核酸酶水 | 至 25 μL | 至 50 μL | – |

3. 短暂离心，将反应体系放置于已经预热的 95℃ PCR 仪上 2min，但不要超过两分钟。
4. 使用推荐的热循环条件进行 PCR：

| 步骤 | 温度 | 时间 | 循环数 |
|---|---|---|---|
| 预变性 | 95°C | 1-2 分钟 | 1 |
| 变性 | 95°C | 30-60 秒 | 25-35 |
| 退火 | 42-65°C | 30 秒 | |
| 延伸 | 72-74°C | 2-4 分钟 /kb | |
| 最后延伸 | 72-74°C | 5 分钟 | 1 |
| 保存 | 4°C | ∞ | |

【使用说明】
1. 酶浓度：我们建议在 50 μL 的反应体系中使用 1.25 units 的 *pfu* DNA 聚合酶。更高的酶浓度会增加由于 *pfu* DNA 聚合酶的 3' 端→ 5' 端的核酸外切活性而引起的引物降解。这也是最后将 *pfu* DNA 聚合酶加入 PCR 混合物中的原因。
2. 模板：使用高质量、纯化的 DNA 模板会大幅提升 PCR 反应的成功率。对于一个 50 μL 反应体系，推荐量的 DNA 模板如下：

| DNA | 用量 |
|---|---|
| 基因组 | 1 ng-1 μg |
| 质粒或病毒 | 1 pg-1 ng |

3. 退火：退火时间通常为 30 s，退火温度基于引物的 Tm 值，一般在 42-65℃之间。退火温度可以通过温度梯度 PCR 进行优化。
4. 引物：寡核苷酸引物一般长度在 20-40 个，GC 含量为 40-60%。如 Oligo 7 软件可被用来设计或分析引物。在 PCR 反应中每个引物的终浓度可以是 0.05~1 μM，通常为 0.1~0.5 μM。
5. 延伸：推荐的延伸温度为 72-74℃。简单样品的延伸时间可以缩短到 1 min/kb。

【参考文献】


1. Fiala G, Stetter K O. Pyrococcus furiosus sp. nov. represents a novel genus of marine heterotrophic archaebacteria growing optimally at 100°C[J]. *Archives of Microbiology*, 1986, 145(1) : 56-61.
2. Lundberg K S, Dan D S, Adams M W W, et al. High-fidelity amplification using a thermostable DNA polymerase isolated from Pyrococcus furiosus. Gene[J]. *Gene*, 1992, 108(1) :1-6.
3. Cline, J., Braman, J.C. and Hogrefe, H.H. (1996) PCR fidelity of Pfu DNA polymerase and other thermostable DNA polymerases. *Nucl. Acids Res.* 24, 3546–51.


【温馨提示】本品不可用于临床治疗。

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