Gene*Taq*™ DNA Polymerase with Colorless Reaction Buffer

合生基因 SyngenTech

Size:

Cat.#	Size
BG10102S	100 units
BG10102M	500 units
BG10102L	2500 units

Concentration: 5 units/μL

Source: *E.coli* strain with a cloned pol gene from Thermus aquaticus YT1.

Storage temperature: -20℃

Exp.: 12 months(-15℃ ~ -25℃)

Applications:

1. Routine PCR amplification of DNA fragments up to 5 kb.
2. PCR product for TA cloning.
3. Colony PCR.

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Description:

Gene*Taq*™ DNA polymerase is a highly thermostable DNA polymerase, that possesses 5' → 3' polymerase activity, and 5'→3' exonuclease activity, but no detectable 3'→5' exonuclease (proofreading) activity. Gene*Taq*™ DNA Polymerase is extremely sensitive and able to amplify trace amounts of DNA in the sample reaction.

Reagent supplied:

Cat.#	Size	Reagent	Quantity
BG10102S	100 units	5×Gene*Taq*™ Colorless Reaction Buffer	1.5 mL
		25 mM $MgCl_2$	1.5 mL
BG10102M	500 units	5×Gene*Taq*™ Colorless Reaction Buffer	4 × 1.5 mL
		25 mM $MgCl_2$	3 × 1.5 mL
BG10102L	2500 units	5×Gene*Taq*™ Colorless Reaction Buffer	20 × 1.5 mL
		25 mM $MgCl_2$	15 × 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 has been detected.
3. Protein Purity (SDS-PAGE): Gene*Taq*™ DNA Polymerase has >99% pure as judged by SDS-PAGE.
4. qPCR for DNA Contamination(*E.coli* Genome): A minimum of 5μL of Gene*Taq*™ 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 of the enzyme is defined as the amount of enzyme that will incorporate 10 nmol of dNTP into acid insoluble material in 30 min at 75 °C.

Storage buffer:

The enzyme is supplied in: 10 mM Tris-HCl (pH7.4@ 25℃), 100 mM KCl, 0.1 mM EDTA, 1 mM DTT, 0.5% (v/v) Tween 20, 0.5% (v/v) NP-40 and 50% (v/v) glycerol.

Protocol:

To prepare several parallel reactions with minimized pipetting errors, prepare a PCR master mix by mixing Nuclease-Free water, buffer, dNTPs, Mg^{2+}, primers and Gene*Taq*™ DNA polymerase. 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.

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	25 μl reaction	50 μl reaction	Final concentration
5 ×Gene*Taq*™ Reaction Buffer	5 μL	10 μL	1 ×
$MgCl_2$ solution, 25 mM	1-4 μL	2-8 μL	1-4 mM
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
GeneTaq DNA Polymerase	0.125 μL	0.25 μL	–
Nuclease-Free water	to 25 μL	to 50 μL	–

3. Gently vortex the samples and spin down.
4. Perform PCR using recommended thermal cycling conditions:

Step	Temperature	Time	Number of cycles
Initial Denaturation	94°C	2-5 min	1
Denaturation	94°C	15-30 s	25-40
Annealing	Tm-5°C	10-30 s	
Extension	68°C	10-60 s/kb	
Final Extension	68°C	5-15 min	1

General guidelines:

1. 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

2. Primers:

Oligonucleotide primers are generally 20-40 nucleotides in length and ideally have a GC content of 40-60%. Software 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.

3. Mg^{2+} and additives:

Mg^{2+} concentration of 1.5-2.0 mM is optimal for most PCR products generated with Gene*Taq*™ DNA Polymerase. The final Mg^{2+} concentration in 1×Gene*Taq*™ Reaction Buffer is 1.5 mM. This supports satisfactory amplification of most amplicons. However, Mg^{2+} can be further optimized in 0.5 or 1.0 mM increments using $MgCl_2$. Amplification of some difficult templates, like GC-rich sequences, may be improved with additives, such as DMSO or formamide.

4. Denaturation:

An initial denaturation of 2 min at 94°C is sufficient for most amplicons from pure DNA templates. For difficult templates such as GC-rich sequences or crude samples, a longer denaturation of 2-8 min at 94°C is recommended prior to PCR cycling to fully denature the template. With colony PCR, an initial 2-5 min denaturation at 94°C is recommended.

5. Annealing:

The annealing step is typically 15-60 s. Annealing temperature is based on the Tm of the primer pair and is typically 45-68°C. Annealing temperatures can be optimized by doing a temperature gradient PCR starting at 5°C below the calculated Tm.

6. Extension:

The recommended extension temperature is 68°C. Extension time are generally 20-30 s/kb for complex, genomic samples, but can be reduced to 10 s/kb for simple templates (plasmid, virus). To determine specific extension time, we recommend 1 min/kb and reduce the extension time until 10 s/kb. 2 min/kb is recommended for crude samples. A final extension of

5 min at 68°C is recommended.

7. Cycle number:
Generally, 25-35 cycles yields sufficient product. Up to 45 cycles may be required to detect low-copy-number templates.

8. PCR product:
The PCR products generated by Gene*Taq*™ DNA Polymerase contain dA overhangs at the 3' end, therefore can be ligated to dT/dU-overhang vectors.

References:

1. Chien A, Edgar D B, Trela J M. Deoxyribonucleic-acid polymerase from extreme thermophile Thermus-aquaticus[J]. *Journal of Bacteriology*, 1976, 127.
2. Kaledin A S, Sliusarenko A G, Gorodetski S I. Isolation and properties of DNA polymerase from extreme thermophylic bacteria Thermus aquaticus YT-1[J]. *Biokhimiia*, 1980, 45(4): 644-651.

Gene*Taq*™ 聚合酶（提供无色反应缓冲液）使用说明书

【产品规格】

货号	规格
BG10102S	100 units
BG10102M	500 units
BG10102L	2500 units

【产品浓度】5 units/μL
【产品来源】重组表达栖热水生菌 DNA 聚合酶基因的大肠杆菌
【储存条件】-20 ℃
【保质期】12 个月（-15℃～-25℃）
【应用范围】
1. 常规 PCR 扩增 5 kb 以内的 DNA 片段
2. 生成用于 TA 克隆的 PCR 产物
3. 菌落 PCR
【产品说明】Gene*Taq*™ DNA 聚合酶是热稳定的 Taq DNA 聚合酶，具有 5'→3' 端 DNA 聚合酶活性和 5'→3' 端核酸外切酶活性，无 3'→5' 端核酸外切酶活性。合生基因的 Gene*Taq*™ DNA 聚合酶对微量的 DNA 样品具有很好的扩增能力。

【随酶提供试剂】

货号	规格	试剂	数量
BG10102S	100 units	5×Gene*Taq*™ 无色反应缓冲液	1.5 mL
		25 mM $MgCl_2$	1.5 mL
BG10102M	500 units	5×Gene*Taq*™ 无色反应缓冲液	4×1.5 mL
		25 mM $MgCl_2$	3×1.5 mL
BG10102L	2500 units	5×Gene*Taq*™ 无色反应缓冲液	20×1.5 mL
		25 mM $MgCl_2$	15×1.5 mL

【质量控制】
1. 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）：用大肠杆菌 16S rRNA 位点通过 Taqman 探针 qPCR 方法，用至少 5 μL 的 Gene*Taq*™ 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 个活性单位是指在 75℃，30 min 内将 10 nmol 脱氧核苷酸合成酸性不溶物所需要的酶量。
【贮存条件】10 mM Tris-HCl (pH7.4 @ 25℃), 100 mM KCl, 0.1 mM EDTA, 1 mM DTT, 0.5% (v/v) 吐温 20, 0.5% (v/v) NP-40, 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，反应缓冲液，dNTPs, Mg^{2+}, 引物和 Gene*Taq*™ DNA 聚合酶制备 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。
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
5× Gene*Taq*™ 反应缓冲液	5 μL	10 μL	1×
dNTP 混合物（各 10 mM）	0.5 μL	1 μL	各 200 μM
25 mM $MgCl_2$	1-4 μL	2-8 μL	1-4 mM
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	可变量	可变量	0.1-100 ng
Gene*Taq*™ DNA 聚合酶	0.125 μL	0.25 μL	–
无核酸酶水	至 25 μL	至 50 μL	–

3. 温和震荡并短暂离心。
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	94℃	2-5 min	1
变性	94℃	15-30 s	25-40
退火	Tm-5℃	10-30 s	
延伸	68℃	10-60 s/kb	
最后延伸	68℃	5-15 min	1

【使用说明】
1. 模板：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

2. 引物：寡核苷酸引物一般长度在 20-40 nt，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。
3. 镁离子和添加成分：1.5-2.0 mM 的镁离子浓度最适于 Gene*Taq*™ DNA 聚合酶大部分 PCR。在一倍 Gene*Taq*™ 混合液中镁离子终浓度为 1.5 mM，满足于大多数的扩增子的扩增。然而，镁离子可以进一步在 0.5 mM 到 1.0 mM 之间优化。在对如高 GC 含量片段这样的困难模板，可以在反应缓冲液体系中适当加入 DMSO 和甲酰胺以提高扩增能力。
4. 变性：94℃ 2 min 预变性即可满足于大部分纯化的 DNA 模板的扩增。对于高 GC 含量序列或者粗样品，推荐使用 2-8 min 的预变性时间来完全变性模板。对于菌落 PCR，推荐使用 2-5 min 的预变性时间。
5. 退火：退火时间通常为 15-60 s，退火温度基于引物的 Tm 值，一般在 45 到 68℃之间。退火温度可以通过低于 Tm-5℃的温度梯度 PCR 进行优化。
6. 延伸：推荐的延伸温度为 68℃。复杂或基因组样品的延伸时间一般为 20-30 s/kb。简单模板（质粒，病毒）可以为 10 s/kb。为确定具体的延伸时间，我们推荐从 1 min/kb 开始降低延伸时间直到 10 s/kb。粗样品推荐使用 2 min/kb。68℃最终延伸推荐使用 5 min。
7. 循环数：一般来说，25 到 35 个循环产生足够的 PCR 产物。最高到 45 个循环可用于检测低拷贝目标。
8. PCR 产物：使用 Gene*Taq*™ DNA 聚合酶产生的 PCR 产物在 3' 端会含有 dA 突出，因此，PCR 产物可以连接到 dT/dU 粘性末端载体上。

【参考文献】

1. Chien A, Edgar D B, Trela J M. Deoxyribonucleic-acid polymerase from extreme thermophile Thermus-aquaticus[J]. *Journal of Bacteriology*, 1976, 127.
2. Kaledin A S, Sliusarenko A G, Gorodetski S I. Isolation and properties of DNA polymerase from extreme thermophylic bacteria Thermus aquaticus YT-1[J]. *Biokhimiia*, 1980, 45(4): 644-651.

【温馨提示】本品仅限于科学研究，不可用于临床治疗。