# T4 DNA Ligase

合生基因 SyngenTech

**Size**:

| Cat#. | Size |
|---|---|
| BG10201S | 20000 U |
| BG10201L | 100000 U |

**Concentration:** 400 units/μL
**Source:** *E.coli* cells with a cloned T4 DNA ligase gene from bacteriophage T4.
**Storge temperature:** -20℃ .
**Exp.:** 12 months(-15℃ ~ -25℃ )
**Applications**:
1. Cloning of restriction fragments
2. Cloning of PCR products
3. Joining of double stranded linkers and adapters to DNA
4. Nick repair in duplex DNA, RNA or DNA/RNA hybrids


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**Description:**
T4 DNA ligase catalyzes the formation of a phosphodiester bond between juxtaposed 5' phosphate and 3' hydroxyl termini in duplex DNA or RNA. With the 10 × T4 DNA Ligase Buffer, the enzyme can ligate DNA sticky or blunt end fragments.
**Reagent Supplied:**

| Cat#. | Size | Reagent | No. |
|---|---|---|---|
| BG10201S | 20000 U | 10 × T4 DNA Ligase Reaction Buffer | 1 mL |
| BG10201L | 100000 U | 10 × T4 DNA Ligase Reaction Buffer | 2 × 1 mL |

**Quality Control:**
1. Protein Purity (SDS-PAGE): T4 DNA Ligase has >99% pure judged by SDS-PAGE.
2. Non-Specific DNase Activity (16 h): The product is tested for non-specific nuclease degradation in a reaction containing a DNA substrate. After incubation for 16 h there is no detectable degradation of the DNA substrate as determined by agarose gel electrophoresis.
3. Endonuclease Activity (Nicking): The product is tested in a reaction containing a supercoiled DNA substrate. After incubation for 4 h, < 5% DNA converted to the nicked form is determined by agarose gel electrophoresis.
4. qPCR for DNA Contamination(*E.coli* Genome): A minimum of 5 μL of T4 DNA Ligase 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 required to reach 50% ligation of 100ng HindIII fragments of λDNA in a total reaction volume of 20 μL in 30 min at 16°C in 1× T4 DNA Ligase Buffer.
**Storage buffer**:
The enzyme is supplied in: 10 mM Tris-HCl (pH7.5 @ 25°C), 50 mM KCl, 0.1 mM EDTA, 1 mM DTT and 50% (v/v) glycerol.
**Protocol**:
1. Thaw the 10 × T4 DNA Ligase Reaction Buffer at room temperature and vortex vigorously to dissolve any precipitated material before use.
2. A molar ratio of 3:1 insert: vector(10-100ng) is recommended for the Reaction of DNA inserts to vectors. To calculate the amount of insert for a 3:1 ratio of vector to insert, use the formula:

$$\text{X ng of insert} = \frac{3 \times \text{insert size (in bp)} \times \text{amount of vector (in ng)}}{\text{vector size (in bp)}}$$

Add the following reagents to an autoclaved, 0.2 mL microcentrifuge tube:

| Reagent | Amount |
|---|---|
| 10 × T4 DNA Ligase Reaction Buffer | 2 μL |
| Linearized vector DNA | 10-100 ng |
| Insert DNA | variable |
| T4 DNA Ligase | 1 μL |
| Nuclease-Free water | to 20 μL |

3. Mix gently and centrifuge briefly.
4. Incubate at room temperature for 10 min or 16°C overnight for sticky ends. Incubate at room temperature for 2 h or 16°C overnight for blunt ends or single base overhang.
5. Use 2-5 μL of the ligation mixture for transform or Store at -20°C.

**Reference:**
Remaut, E., Tsao, H., Fiers, W,. Improved plasmid vectors with a thermoinducible expression and temperature-regulated runaway replication [J]. *Gene*, 1983, 22(1) :103-113.

Research only, not for therapeutic or diagnostic purposes.

# T4 DNA 连接酶使用说明书

【产品名称】T4 DNA 连接酶

【产品规格】

| 货号 | 规格 |
|---|---|
| BG10201S | 20000 U |
| BG10201L | 100000 U |

【产品浓度】400 units/μL

【产品来源】重组表达 T4 噬菌体中 T4 DNA Ligase 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. 限制性酶切片段的克隆
2. PCR 产物克隆
3. 将双链 linkers 或 adapters 连接到 DNA 上
4. dsDNA、dsRNA 或 DNA/RNA 杂交链的切刻修复

【产品说明】

T4 DNA 连接酶催化双链的 DNA 或 RNA 中相邻的 5'- 磷酸基团和 3' 羟基末端形成磷酸二酯键。在含有 10×T4 DNA 反应缓冲液的环境下，T4 DNA 连接酶可以链接 DNA 的粘性末端和平末端。

【随酶提供试剂】

| 货号 | 规格 | 试剂 | 数量 |
|---|---|---|---|
| BG10201S | 20000 U | 10×T4 DNA 反应缓冲液 | 1 mL |
| BG10201L | 100000 U | 10×T4 DNA 反应缓冲液 | 2×1 mL |

【质量控制】

1. 蛋白纯度（SDS-PAGE）: SDS-PAGE 检测纯度 >99%
2. 非特异性核酸酶活性：将 T4 DNA 连接酶和 DNA 底物混合孵育 16 个小时，所得产物经过凝胶电泳未检测到 DNA 降解。
3. 内切酶（切刻）活性：将 T4 DNA 连接酶与超螺旋结构的 DNA 混合进行孵育 4h，< 5% 的超螺旋 DNA 转化为有切刻形式的 DNA。

【核酸污染】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 T4 DNA 连接酶来检测，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 个单位指在 16℃，20 μL T4 DNA 反应缓冲液反应缓冲液条件下，30 分钟能使 50% 的经 Hind III 消化的 100ng λ DNA 片段所需的酶量。

【贮存条件】10 mM Tris-HCl (pH 7.5 @ 25°C), 50 mM KCl, 0.1 mM EDTA, 1 mM DTT 和 50%(w/v) 甘油。

【操作步骤】

1. 将 10×T4 DNA 反应缓冲液放置于室温中，请在使用前充分混匀。
2. 将 10~100 ng 的线性化载体和大于等于 3 倍摩尔量的插入片段进行混合，建议使用公式：

$$X\ ng\ of\ insert = \frac{3 \times insert\ size\ (in\ bp) \times amount\ of\ vector\ (in\ ng)}{vector\ size\ (in\ bp)}$$

按下表要求在 0.2 mL 离心管中加入各种试剂：
具体反应体系如下：

| 试剂 | 含量 |
|---|---|
| 10×T4 DNA 反应缓冲液 | 2 μL |
| 线性 DNA 载体 | 10~100 ng |
| 插入 DNA 片段 | 可变量 |
| T4 DNA Ligase | 1 μL |
| 无核酸酶水 | 至 20 μL |

3. 混合均匀并短暂离心。
4. 如果是粘性末端的连接则在 16℃反应过夜或是在室温反应 10min，如果是平末端或是单碱基的粘性末端，则 16℃反应过夜或是在室温中反应 2h。
5. 取 2-5 μL 产物转化或者放置于 -20℃进行储存。

【参考文献】

Remaut, E., Tsao, H., Fiers, W,. Improved plasmid vectors with a thermoinducible expression and temperature-regulated runaway replication [J]. *Gene*, 1983, 22(1) : 103-113.

【温馨提示】本品仅限于科学研究，不可用于临床治疗。