Quick Ligase™ Kit

合生基因 SyngenTech

Size:

Cat#.	Size
BG10202S	30 reactions
BG10202L	150 reactions

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:

Quick Ligase™ catalyzes the formation of a phosphodiester bond between juxtaposed 5' phosphate and 3' hydroxyl termini in duplex DNA or RNA. With the 2 × Quick ligase™ Buffer, the enzyme can ligate DNA sticky or blunt end fragments in 5 min at room temperature.

Reagent Supplied:

Cat#.	Size	Reagent	No.
BG10202S	30 reactions	2 × Quick Ligase™ Reaction Buffer	1 mL
BG10202L	150 reactions	2 × Quick Ligase™ Reaction Buffer	2 × 1 mL

Quality Control:

1. Protein Purity (SDS-PAGE): Quick Ligase™ is >99% pure as 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 Quick 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.

Storage buffer:

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 2 × Quick 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 to 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
2 × Quick Ligase™ Reaction Buffer	10 μL
Linearized vector DNA	10-100 ng
Insert DNA	variable
Quick Ligase™	1 μL
Nuclease-Free water	to 20 μL

3. Mix gently and centrifuge briefly.
4. Incubate at room temperature for 5 min.
5. Use 2-5 μL of the ligation mixture for transform or store at -20°C.
6. Do not heat inactivate. Heat inactivation dramatically reduces transformation efficiency.

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.

快速 DNA 连接酶使用说明书

【产品名称】快速 DNA 连接酶

【产品规格】

货号	规格
BG10202S	30 reactions
BG10202L	150 reactions

【储存条件】-20 ℃

【保质期】12 个月（ -15℃～-25℃ ）

【应用范围】

1. 5 分钟快速连接粘性末端或平末端。室温下即可进行连接反应
2. PCR 产物克隆
3. T/A 克隆
4. dsDNA 切刻修复

【产品说明】

Quick Ligase ™催化双链的 DNA 或 RNA 中相邻的 5'- 磷酸末端和3'羟基末端形成磷酸二酯键。Quick Ligase ™在室温(25℃) 反应 5 min 即可完成 DNA 粘性末端或平齐末端的连接反应。

【随酶提供试剂】

货号	规格	试剂	数量
BG10202S	30 reactions	2×Quick Ligase™ 反应缓冲液	1 mL
BG10202L	150 reactions	2×Quick Ligase™ 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 Quick Ligase ™和双链 DNA 和单链 DNA 混合孵育 16 h，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 Quick Ligase ™与超螺旋结构的 DNA 混合进行孵育 4 h，<5% 的超螺旋 DNA 转化为了有切刻形式的 DNA。
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。

【贮存条件】

10 mM Tris-HCl (pH 7.5 @ 25℃), 50 mM KCl, 0.1 mM EDTA, 1 mM DTT 和 50%(w/v) 甘油。

【 操作步骤 】

1. 将 2× 快速 DNA 连接反应缓冲液放置于室温中，请在使用前充分混匀。
2. 将 10-100ng 的载体和大于等于 3 倍分子量的插入片段进行混合，并进入 ddH2O 稀释至 10 μL。建议使用公式：

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

按下表要求在 0.2 mL 离心管中加入各种试剂：

试剂	数量
2× 快速 DNA 连接反应缓冲液	10 μL
线性 DNA 载体	10-100 ng
插入 DNA 片段	variable
快速 DNA 连接酶	1 μL
无核酸酶水	to 20 μL

具体反应体系如下：

3. 混合均匀并短暂离心。
4. 室温（ 25℃ ）放置反应 5 min。
5. 将反应体系放置于冰上，然后取 2~5 μL 产物转化或者放置于 -20℃进行储存。
6. 不要进行热失活，热失活后将影响转化效率。

【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。