QIAGEN

Quick-Start Protocol August 2023

T4 DNA Ligase MBG

The T4 DNA Ligase MBG (cat. nos. EN11-050 and EN11-250) is an ATP-dependent recombinant enzyme isolated from *E. coli* strain. T4 DNA Ligase MBG catalyzes the formation of a phosphodiester bond between juxtaposed 5'-phosphate and 3'-hydroxyl termini in duplex DNA or RNA. It will join both blunt-ended and cohesive-ended restriction fragments of DNA, as well as repair single-stranded nicks in duplex DNA, RNA, or DNA/RNA hybrids.

The T4 DNA Ligase MBG and its components should be shipped on dry ice, and stored at –20°C.

Further information

- Safety Data Sheets: **www.qiagen.com/safety**
- Technical assistance: **support.qiagen.com**

Notes before starting

- The 10x Ligation Buffer and ATP Solution should be thawed and resuspended at room temperature.
- For blunt-end ligations, use higher quantities of both the vector and the insert DNA.
- The enzyme is inhibited by >200 mM NaCl or KCl concentrations.
- Inactivate enzyme at 65°C for 10 minutes or at 70°C for 5 minutes.
- Enzyme concentration is 5U/µL
- Polyethylene glycol (PEG) addition greatly increases the ligation efficiency of blunt-end DNA ligation. The recommended concentration of PEG in the ligation reaction mixture is 5% (w/v) (high concentration of PEG significantly reduces the transformation efficiency of electrocompetent cells).

- One (Weiss) unit of T4 DNA Ligase MBG catalyzes the conversion of 1 nmol of ^{32}P from pyrophosphate into Norit-adsorbable material in 30 minutes at 37°C.
- One Weiss unit is equivalent to approximately 200 cohesive end units.
- Using a 3–10 molar excess of insert DNA over vector DNA is recommended.
- To calculate the optimal amounts of insert DNA in a ligation reaction, use the following equation:

$$\text{ng of insert} = \frac{\text{ng of vector x kb size of insert x molar ratio insert : vector}}{\text{kb size of vector}}$$

Procedure

1. Refer to Table 1 for the list of reaction reagents, and add them accordingly to a sterile nuclease-free Eppendorf tube placed on ice in a freezing rack.

   Note: The reaction agents should be added according to the order indicated in Table 1.

Table 1. Order on how reaction agents should be added

Component	Volume
Vector DNA	x µL (20–50 ng)
Insert DNA	y µL (3–10 molar excess over vector)*
10x Ligation Buffer	2 µL
ATP Solution (25 mM)	0.4–0.8 µL
T4 DNA Ligase MBG	1 µL
Nuclease-free Water	Up to 20 µL

* A lower ratio will result in a less efficient ligation; a higher ratio will incite multiple insertions. This data is intended for use as a guide only; conditions will vary from reaction to reaction and may need optimization.

2. Mix gently and spin briefly.
3. For cohesive (sticky) ends, incubate at 20–25°C for 30 min to 1 h.

   For blunt ends, incubate at 20–25°C for 1–2 h.
4. Cool the samples on ice and transform 1–5 µL of the reaction mixture into 50 µL competent cells.

Document Revision History

Date	Changes
08/2023	Initial release

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