

## INSTRUCTIONS FOR USE

---

**Product Name: T4 DNA Ligase****Catalog # T401-E24F**

---

**Step 1.** In a centrifuge tube add the following reaction components:

2 $\mu$ L	10x Ligase Buffer
1 $\mu$ L	T4 DNA Ligase (5 U/ $\mu$ L)
Molar ratio of fragment to carrier is between 3:1 and 5:1	Insert Fragment
50-100 ng	DNA Template
Up to 20 $\mu$ L	ddH <sub>2</sub> O

- Dephosphorylate vectors that have a flat-end vector to prevent self-ligation.
- 2  $\mu$ L of 50% PEG 4000 can be added to the 20  $\mu$ L reaction to improve efficiency.

**Step 2.** Perform ligation overnight at 16°C.

**Step 3.** Store at -20°C for up to 2 years. Aliquot to smaller amounts to avoid free-thaw.