**USB® T4 DNA Ligase**

*Product numbers 70005/X/Y, 70042/X*

T4 DNA Ligase catalyzes the formation of a phosphodiester bond between juxtaposed 5'-phosphoryl and 3'-hydroxyl termini in duplex DNA. It repairs single-stranded nicks in duplex DNA and will join both blunt-ended and cohesive-ended restriction fragments of duplex DNA.

**Properties:**
- Optimum pH: 7.5–7.8
- Optimum temperature: 16°C
- Requirement for divalent cation: Mg²⁺, Mn²⁺
- Optimum Mg²⁺ concentration: 10 mM
- Requirement for cofactor: ATP
- Sulfhydryl requirement: β-ME, DTT
- Stimulators: Spermine (0.5 to 1 mM), Spermidine (0.5 to 1 mM)
- Inhibitors: Na (>0.2 M), K (>0.2 M)
- Inactivation: By heating at 65°C for 10 minutes

**10X T4 DNA Ligase Reaction Buffer**
- (PN 70087, included with enzyme): 660 mM Tris-HCl, pH 7.6, 66 mM MgCl₂, 100 mM DTT, 660 µM ATP

**T4 DNA Ligase Dilution Buffer**
- (Included with enzyme): 20 mM Tris-HCl, pH 7.6, 1 mM EDTA, 5 mM DTT, 60 mM KCl, 50% glycerol

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T4 DNA Ligase and 10X Reaction Buffer have been functionally tested in the following protocol:

**Ligation protocol**

Each ligation reaction should contain 20–40 ng of vector DNA and maintain a vector to insert ratio of 1:3–10.

1. Combine the following:
   - Vector DNA (20–40 ng) __ µl
   - Insert DNA (3–10 fold molar excess) __ µl
   - Reaction Buffer, 10X __ µl
   - Water __ µl
   - T4 DNA Ligase __ µl (1 unit)

   **Total** 50 µl

2. Mix and incubate at 16°C for 16 hours or at room temperature for 1–2 hours.

3. Transform immediately or store at -20°C for later transformation. Use 1–5 µl of ligation reaction per 50 µl of competent cells.

**Note:** For blunt-end ligations we recommend using the higher quantities of both vector and insert DNA.

To stimulate the blunt-end ligation reaction, 150–200 mM NaCl and 5% PEG can be added.