**ElectroLigase**

50 reactions | Lot: 0161608
RECOMBINANT | Store at −20°C | Exp: 8/18

**Description:** ElectroLigase combines T4 DNA ligase and an optimized, ready-to-use 2X reaction buffer containing a proprietary ligation enhancer and no PEG. This combination is specifically formulated to promote robust ligation of all types of DNA ends (blunt, sticky, TA). It is directly compatible, without desalting or purification, with electrocompetent cells used for transformation by electroporation. No thawing of the buffer is required as it maintains a liquid state during storage at −20°C, thereby simplifying reaction set-up. By providing an optimized ratio of enzyme and buffer components, users are able to rapidly ligate all types of DNA ends using a short incubation time at room temperature. Ligation reactions with blunt ends (A) or TA (B) substrates were set-up with ElectroLigase. Incubations were performed at 25°C for the indicated time and used to transform electrocompetent E. coli by electroporation. Number of transformants (colonies x plating dilution factor) was graphed as a function of ligation incubation time.

**Reaction Conditions:**
- 1X ElectroLigase Reaction Buffer with DNA substrates and 1 μl ElectroLigase
- Electrocompetent strains of E. coli®

**Ligation Protocol for Subcloning:**
1. Transfer ElectroLigase and ElectroLigase Reaction Buffer to ice prior to reaction set up. Mix tubes by finger-flicking before use.
2. Combine 20–100 ng of vector* with a 3-fold molar excess of insert and adjust volume to 5 μl with dH₂O.
3. Add 5 μl of ElectroLigase Reaction Buffer and 1 μl of ElectroLigase and pipet up and down 7–10 times to mix.
4. Incubate ligation reaction at room temperature (25°C) for 30 minutes.
5. Inactivate the ligase by incubating the reaction at 65°C for 15 minutes.
6. Chill sample on ice (if to be used within a few hours) or store at −20°C.

* In-house testing has demonstrated that maximal transformation efficiency is achieved using between 20–100 ng of vector (blunt or sticky, including T-vectors) and a corresponding 3-fold molar excess of the insert to be ligated into the vector.

**Transformation Protocol:**
Electrocompetent strains of E. coli (commercially available or prepared by user) can be transformed by ligation products prepared using ElectroLigase. Chemically competent cells are also compatible, but for maximum performance with chemically competent cells, please consider using the Blunt/TA Ligase Master Mix (NEB M0367). The following protocol is recommended by NEB. Other protocols can be used but the volume of ligation reaction used should not exceed 5 μl per 50 μl cells.
1. Thaw competent cells on ice.
2. Aliquot 40 μl of cells into a 1.5 ml microcentrifuge tube on ice.
3. Add 2 μl of the ligation reaction to the cells and mix by finger-flicking. Do not vortex the tube.
4. Transfer DNA/competent cell mixture to a pre-chilled electroporation cuvette and follow the manufacturers recommendations for electroporation (e.g. 2500 V, 200 μF, 2 mm gap cuvette).
5. Add 760 μl recovery media (e.g. SOC) to the cuvette, mix, transfer the transformed cells to a culture tube and incubate for one hour at 37°C with shaking (200–250 rpm).
6. Spread 50 μl of the outgrowth (undiluted or diluted 1:5 with recovery media) onto appropriate antibiotic selection plates and incubate overnight at 37°C.

**Source:** Purified from an E. coli strain containing a recombinant gene encoding T4 DNA Ligase.

**Applications:**
- Vector construction
- Linker ligation
- Fragment assembly
- Library construction
- TA cloning

**Quality Controls:**
- ElectroLigase is tested for transformation efficiency using the following protocol.
- LITMUS® 28 vector is cut with either EcoRV (blunt) or HindIII (cohesive), treated with calf intestinal phosphatase and gel purified. Blunt inserts from a HaellI digest of φX174 DNA and cohesive inserts from a HindIII digest of λ DNA are ligated into the respective vectors at a 3:1 insert:vector molar ratio using the ElectroLigase Protocol. Ligation products are transformed as described.
- Each lot exceeds the following standards for efficiency (transformants/μg):
  - Recircularization: Cohesive ends (> 1 x 10⁷), Blunt ends (> 1 x 10⁸), Uncut vector (1 x 10⁸)
  - Insertion: Cohesive ends (> 1 x 10⁷), Blunt ends (> 2.5 x 10⁸)

**Basic Protocol**
1. Transfer ElectroLigase and ElectroLigase Reaction Buffer to ice prior to reaction set up. Mix tubes by finger-flicking before use.
2. Combine 20–100 ng of vector* with a 3-fold molar excess of insert and adjust volume to 5 μl with dH₂O.
3. Add 5 μl of ElectroLigase Reaction Buffer and 1 μl of ElectroLigase and pipet up and down 7–10 times to mix.
4. Incubate ligation reaction at room temperature (25°C) for 30 minutes.
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* In-house testing has demonstrated that maximal transformation efficiency is achieved using between 20–100 ng of vector (blunt or sticky, including T-vectors) and a corresponding 3-fold molar excess of the insert to be ligated into the vector.

**Usage Notes:**
- Cells: Competent cells can vary by several logs in their competence. Perceived ligation efficiency directly correlates with the competence of the cells used for transformation. Always transform uncut vector as a control for comparison purposes.
- DNA: Purified DNA for ligations can be dissolved in dH₂O (Milli-Q® water or equivalent is preferable); TE or other dilute buffers also work well. For optimum ligation, the amount of vector DNA should be 20–100 ng and the insert should be added at a 3-fold molar excess. For ligation volumes greater than 10 μl, increase the volume of ElectroLigase Reaction Buffer such that it remains 50% of the reaction. Insert:vector ratios between 2 and 6 are optimal for single insertions. Ratios below 2:1 result in lower ligation efficiency. Ratios above 6:1 promote multiple inserts. If you are unsure of your DNA concentrations, perform multiple ligations with varying ratios.

**Time and Temperature:**
- Most ligations performed using ElectroLigase reach an end point at 60 minutes or less when performed between 4–37°C. Incubation beyond this time provides no additional benefit. Our recommendation for a 25°C (room temperature) incubation was chosen after evaluation of performance at 4°C, 16°C, 25°C, and 37°C. Most conditions reached at least 50% performance within 30 minutes.

**Biology:** Some DNA sequences are not easy to clone. Sequences that form structures, including inverted and tandem repeats, are selected against by E. coli. Some recombinant proteins are not well tolerated by E. coli and can result in poor transformation or small colonies.

(see other side)
Companion Products Sold Separately:
Blunt/TA Ligase Master Mix
#M0367S 50 rxns
#M0367L 250 rxns

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