ElectroLigase™

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M0369S 001120813081

M0369S

50 reactions **Lot: 0011208**

RECOMBINANT **Store at –20°C** **Exp: 8/13**

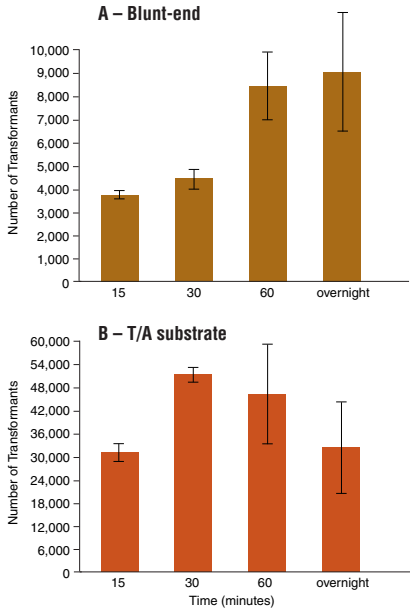
Description: ElectroLigase combines T4 DNA ligase and an optimized, ready-to-use 2X reaction buffer containing a proprietary ligation enhancer and no PEG. This combination is specifically formulated to promote robust ligation of all types of DNA ends (blunt, sticky, TA). It is directly compatible, without 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 applying a short incubation time at room temperature. Ligations for subcloning can be carried out in small volumes with low concentrations; allowing users to conserve precious DNA samples. These reactions can be pipetted directly, without purification or dilution, to transform many strains of electrocompetent *E. coli***.

* Freezers vary in their actual internal temperatures. Our testing demonstrates that the enzyme and buffer remain liquid at –20°C.

**ElectroLigase is also compatible with chemically competent strains of *E. coli*. Performance is generally around 50% efficiency, when compared to the Blunt/TA Ligase Master Mix (NEB #M0367).

Applications:

- Vector construction
- Linker ligation
- Fragment assembly
- Library construction
- TA cloning



Ligations performed using ElectroLigase reach an endpoint at 60 minutes or less. Reactions containing equal amounts (20 ng vector and 3-fold molar excess of insert) of blunt (A) or T/A (B) vector/insert pairs were set up using ElectroLigase and incubated for the times shown. After heat inactivation of the ligase, 2 µl of each reaction were withdrawn and directly used to transform NEB 10-beta Electrocompetent E. coli (NEB #C3020). 50 µl aliquots of the outgrowth (diluted, in some cases) was plated onto selective pates and incubated overnight at 37°C. Colonies were counted, adjusted for plating dilution, and graphed.

Reaction Conditions: 1X ElectroLigase Reaction Buffer with DNA substrates and 1 µl ElectroLigase in an 11 µl reaction volume incubated at 25°C for 30 minutes.

Heat Inactivation: Yes

Quality Controls

The 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 HaeIII digest of ϕX174 DNA and cohesive inserts from a HindIII of 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 10^7), Blunt ends (> 1 x 10^7), Uncut vector (1 x 10^8)

Insertion: Cohesive ends (> 1 x 10^7), Blunt ends (> 2.5 x 10^6)

Protocols

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 dH_2O.
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 30–60 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 with 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 reaction 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 Ω, 25 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.

Typical Results:

Transformation efficiencies around 3 x 10^6 cfu/µg are typically achieved for recombinant blunt-end vectors (vector + insert), using cells with a 5 x 10^9 calculated efficiency with uncut DNA. Results for TA cloning and standard cohesive end (4 bp overhang) cloning produce even higher numbers, often over 10^7 cfu/µg. This corresponds to several hundred colonies on a plate when 50 µl of the outgrowth is plated at a 1:5 dilution. As with all ligation and transformation protocols, many factors affect the calculated transformation efficiency, including purity and integrity of DNA ends, competence of the cells being transformed, media choices, incubation temperatures and times and biological effects (intact ORF in high-copy vector, toxic genes, etc.).

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 dH_2O (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 11 µl, increase the volume of ElectroLigase Reaction Buffer accordingly. 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)

CERTIFICATE OF ANALYSIS

Companion Products Sold Separately:

Blunt/TA Ligase Master Mix

#M0367S	50 rxns
#M0367L	250 rxns