

Instant Sticky-end Ligase Master Mix



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50 reactions (250 µl vol) Lot: **0031305**
RECOMBINANT Store at -20°C Exp: 5/14

Description: Instant Sticky-end Ligase Master Mix is a ready-to-use 2X solution of T4 DNA ligase and a proprietary ligation enhancer in an optimized reaction buffer. Specifically formulated to rapidly ligate cohesive-end (2–4 bp) substrates and improve transformation, the mix simplifies reaction set-up and provides an optimized ratio of enzyme and buffer components. No thawing of the master mix is required as it maintains a liquid state during storage at -20°C* and no incubation time is necessary to achieve ligation efficiencies sufficient for successful sub-cloning of sticky-end substrates. Just add the master mix to DNA with compatible ends, mix and transform; thereby reducing the valuable time needed for routine ligations. Ligations for subcloning can be carried out in small volumes with low concentrations, allowing users to conserve precious DNA samples, and be used directly to transform many strains of chemically competent *E. coli* without dilution.

* Freezers vary in their actual internal temperature. Our testing demonstrates that the master mix is liquid at -20°C. Freeze-thaw testing at -70°C has confirmed that the performance after 20 freeze/thaw cycles is close to the original mix.

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

Applications:

- Vector construction
- Fragment assembly
- Library construction

Reaction Conditions: 1X Instant Sticky-end Ligase Master Mix with DNA substrates in a 10 µl reaction volume. A 10 µl reaction contains 1800 cohesive end units of T4 DNA Ligase.

Heat Inactivation: No

Quality Controls

The Instant Sticky-end Ligase Master Mix is tested for transformation efficiency using the following protocol.

LITMUS 28 vector is cut with HindIII (cohesive), treated with calf intestinal phosphatase and gel purified. Cohesive inserts from a HindIII of digest of λ DNA are ligated into the vector at a 3:1 insert:vector ratio using the Instant Sticky-end Ligase Master Mix Protocol. Ligation products are transformed as described.

Each lot exceeds the following standards:

	Efficiency (transformants/µg)	
	Recircularization	Insertion
Cohesive ends	> 1 x 10 ⁷	> 2.5 x 10 ⁶
Uncut vector	> 1 x 10 ⁸	

Protocols

Ligation Protocol for Subcloning:

1. Transfer master mix to ice prior to reaction set up. Mix tube 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 Instant Sticky-end Ligase Master Mix, mix thoroughly by pipetting up and down 7–10 times, and place on ice. The sample is now ready to be used for transformation.
5. Use for transformation or store at -20°C.
6. **Do not heat inactivate.**

Heat inactivation dramatically reduces transformation efficiency.

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

Transformation Protocol:

Chemically competent strains of *E. coli* (commercially available or prepared by user) can be transformed by ligation products prepared using the Instant Sticky-end Ligase Master Mix. Electrocompetent cells are not compatible. Users of competent cells from other vendors may need to dilute ligation reactions 4-fold, prior to transformation, in order to achieve maximum transformation efficiency. Not all cells from other vendors will benefit from this additional step. 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 50 µl of cells into a 1.5 ml microcentrifuge tube.
3. Add 2 µl of the ligation reaction to the cells and mix by finger-flicking. Do not vortex the tube.
4. Incubate the tube on ice for 30 minutes. Do not mix.
5. Heat shock at 42°C for 30 seconds, return tube to ice for 2 minutes.
6. Add 950 µl recovery media (e.g. SOC) to the tube and incubate for one hour at 37°C with rotation or shaking (200–250 rpm).
7. Spread 100 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 2 x 10⁶ cfu/µg are typically achieved for recombinant cohesive-end substrates (vector + insert), using cells with a 7 x 10⁸ calculated efficiency with uncut DNA. This corresponds to several hundred colonies on a plate when 100 µl of a 1 ml 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.

Electroporation: While electroporation can dramatically increase transformation efficiency, Instant Sticky-end Ligase Master Mix is not directly compatible with transformation by electroporation. It is necessary to reduce the PEG concentration. We recommend purification of the ligated DNA by spin column.

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 Instant Sticky-end Ligase Master Mix 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: Ligations performed using the Instant Sticky-end Ligase Master Mix are immediately ready to be used to transform *E. coli*. If it is necessary to incubate the reaction prior to transformation, performance is generally good between 4–25°C. Incubation can be performed for up to one hour at the chosen temperature without negatively impacting the performance of the mix.

Blunt and TA ends can also be ligated by this mix but require a 5–15 minute incubation time to reach our internal performance standards. The "instant" ligation criteria were met with cohesive ends of 2–4 bp in length.

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.



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