Blunt/TA Ligase Master Mix

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50 reactions (250 µl vol) **Lot: 0091409**

RECOMBINANT Store at –20°C **Exp: 9/15**

Description: Blunt/TA Ligase Master Mix is a ready-to-use 2X solution of T4 DNA Ligase, proprietary ligation enhancer, and optimized reaction buffer. This master mix is specifically formulated to improve ligation and transformation of both blunt-end and single-base overhang substrates. The master mix format simplifies reaction set-up, ensures an optimized ratio of enzyme and buffer components, and yields robust, rapid ligation of all types of DNA ends using a short incubation time at room temperature. No thawing is necessary as it remains liquid during storage at –20°C.* Ligations for subcloning can be carried out in small volumes with low DNA concentrations, allowing users to conserve precious DNA samples and directly 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 is unchanged after 20 freeze/thaw cycles.

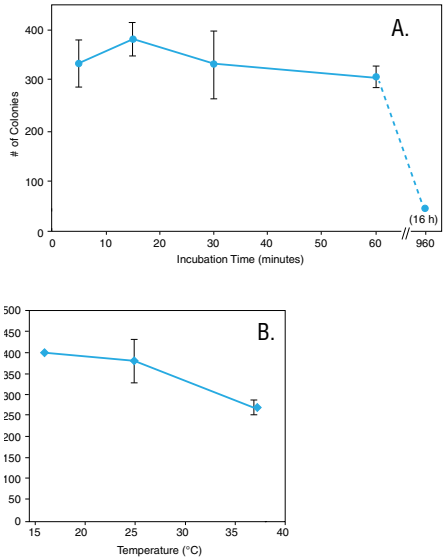
Source: Purified from an *E. coli* strain containing a recombinant gene encoding T4 DNA Ligase.

Applications:

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

Reaction Conditions: 1X Blunt/TA Ligase Master Mix with DNA substrates in a 10 µl reaction volume incubated at 25°C. A 10 µl reaction contains 1,800 cohesive end units of T4 DNA Ligase.

Heat Inactivation: No



Ligation reactions with single-base overhang vector and insert were set-up using the Blunt/TA Ligase Master Mix and incubated for different times at 25°C (A) or at different temperatures for 15 minutes (B). Two microliters of each reaction were used to transform a 50 µl aliquot of NEB 10-beta Competent E. coli (NEB #C3019). Transformants resulting from triplicate plating 50 µl of a 1:5 dilution of the outgrowth were counted and graphed. The results indicate that the Blunt/TA Ligase Master Mix works well at 25°C, and is complete in 15 minutes.

Quality Controls

The Blunt/TA Ligase Master Mix is tested for transformation efficiency using the following protocol.

LITMUS 28 vector is cut with EcoRV (blunt), treated with calf intestinal phosphatase and gel purified. Blunt inserts from a HaeIII digest of ϕX174 DNA are ligated into the vector at a 3:1 insert:vector ratio using the Blunt/TA Ligase Master Mix Protocol. Ligation products are transformed as described.

Each lot exceeds the following standards:

	Efficiency (transformants/µg)	
	Recircularization	Insertion
Blunt 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 dH_2O.
3. Add 5 µl of Blunt/TA Ligase Master Mix and mix thoroughly by pipetting up and down 7-10 times or by finger-flicking.
4. Incubate at room temperature (25°C) for 15 min, place on ice.
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 (blunt or sticky, including T-vectors) 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 Blunt/TA 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, then place on 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 10^6 cfu/µg are typically achieved for recombinant blunt-end vectors (vector + insert), using cells with a 7 x 10^8 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 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, Blunt/TA 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 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 10 µl, increase the volume of Blunt/TA 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.

(see other side)

Time and Temperature: Most ligations performed using the Blunt/TA Ligase Master Mix 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 15 minutes. Shorter times can also be used.

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.