50 reactions (250 μl vol) Lot: 0011207
RECOMBINANT Store at –20°C Exp: 7/13

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 single incubation time at room temperature. No thawing is necessary as it remains liquid during storage at –20°C. Ligation reactions 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 is unnecessary as it remains liquid during storage at –20°C. Ligation reactions 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.

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

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:

<table>
<thead>
<tr>
<th>Efficiency (transformants/μg)</th>
<th>Recirculation</th>
<th>Insertion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blunt ends</td>
<td>&gt; 1 x 10⁷</td>
<td>&gt; 2.5 x 10⁶</td>
</tr>
<tr>
<td>Uncut vector</td>
<td>&gt; 1 x 10⁸</td>
<td></td>
</tr>
</tbody>
</table>

**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 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. Electroporation is not directly compatible with this product. 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 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:** When 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 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 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.

**Typical Results:**

Transformation efficiencies around 2 x 10⁶ cfu/μg are typically achieved for recombinant blunt-end vectors (vector + insert), using cells with a 7 x 10⁵ calculated efficiency with undig DNA. Results for TA cloning and standard cohesive end (4 bp overhang) cloning produce even higher numbers, often over 10⁷ 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.).
**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.

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