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Blunt/TA Ligase Master Mix

Catalog Number: E103-1, E103-2

Table 1. Kit Components and Storage

Kit Component	E103-1 (50 rxn)	E103-2 (250 rxn)	Storage	Stability
2× Blunt/TA Ligase Master Mix	250 µL	1.25 mL	-20 °C, avoid repeated free-thaw	The product is stable for at least 6 months when stored as directed.

Product Description

Blunt/TA Ligase Master Mix is a ready-to-use 2× solution, which 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.

Application

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

Ligation Protocol

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.

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.

1. Thaw competent cells on ice.

2. Chill approximately 5 ng (2 µL) of the ligation mixture in a 1.5 mL microcentrifuge tube.
3. Add 50 µL of competent cells to the DNA and mix gently by pipetting up and down.
4. Incubate on ice for 30 minutes. Do not mix.
5. Heat shock for 30 seconds at 42°C, chill on ice for 2 minutes.
6. Add 950 µL of recovery media (e.g. SOC) to the tube and incubate at 37°C for 1 hour with rotation or shaking.
7. Spread 100 µL of the out growth (undiluted or diluted 1:5 with recovery media) onto the appropriate antibiotic selection plates.
8. Incubate overnight at 37°C.