2X Universal Ligation Mix

Product #: Bi2M-2xULM
Quantity: 50 reactions

Product Description

- **Composition:** Ready-to-use 2x master mix with T4 DNA Ligase and reaction buffer.
- **Function of T4 DNA Ligase:**
  - Catalyzes binding of phosphodiester bonds between 5'-P and 3'-OH ends of sticky or blunt-ended double-stranded DNA or RNA.
  - Repairs single-stranded nicks in double-stranded DNA, RNA, and DNA/RNA hybrids.
- **Advantages:**
  - Optimized premixed reaction buffer for efficient and easy operation.
  - Suitable for transforming various chemically competent cells.
- **Reaction Conditions:** Incubate at 25°C for 5 minutes.

Storage

- Store at -20°C.

Components Provided

2X Universal Ligation Mix	250µL

Important Notes (PLEASE READ CAREFULLY)

1. Prepare the reaction system on ice.
2. Gel purify the vector and target fragments, then check their quality and concentration by electrophoresis. If concentrations are low, omit water. Scale up the reaction system to 20 µL if the combined volume of vector and insert exceeds 5 µL.
3. Use a molar ratio of vector to insert between 1:3 and 1:10.

4. For electroporation transformation, purify the ligation product using the column method or ethanol precipitation.
5. Before use, take out the 2X Universal Ligation Mix, and return it to -20℃ immediately after use. Aliquot to minimize repeated freeze-thaw cycles.
6. Dephosphorylate the blunt-end vector (recommended Cat **#Bi2M-AP**) to prevent self-circularization when ligating with DNA fragments.
7. Wear a lab coat and disposable gloves during the procedure.

Protocol

1. Prepare the reaction system as described.

COMPONENT	FINAL VOLUME/AMOUNT
2X Universal Ligation Buffer	1μL
DNA Vector	μL
DNA Insert	μL
Nuclease Free Water	To 10μL

2. Mix gently and centrifuge the liquid to the bottom of the tube.
3. Reaction conditions:
    A. Sticky and Blunt End Ligation Reaction
    B. Sticky end ligation: incubate at 25°C for 5-30 minutes.
    C. Blunt end ligation: incubate at 25°C for up to 2 hours or at 4°C overnight.
4. Ligation Product Transformation
    A. Remove competent cells (e.g., E. coli DH5α, E. coli Top10) from the -80°C freezer and thaw on ice.

B. Add the ligation reaction sample to the competent cells, gently mix by stirring the bottom of the tube with fingers, and place on ice for 30 minutes.
C. Heat shock the cells in a 42°C water bath for 90 seconds, then immediately place on ice for 2-5 minutes.
D. Add 900 µL of sterile SOC or LB medium to the tube, mix, and incubate the tube on a shaker at 220 rpm at 37°C for 1 hour to recover the bacteria (alternatively, incubate in a 37°C incubator for 1 hour).
E. Plate different volumes of transformed competent cells onto LB agar plates containing the appropriate antibiotics. Spread the cells evenly, allow the liquid to absorb, and then incubate the plates upside down at 37°C overnight.

5. Positive Clone Identification
   A. Pick single colonies from the plate and perform colony PCR for identification.
   B. Alternatively, culture the colonies, extract plasmids, and perform digestion or PCR for identification.
   C. Direct sequencing and analysis of extracted plasmids can also be used for identification.