MCLAB
Molecular Cloning Laboratories

User Manual

Version 3.0

Product name: T3 DNA Ligase

Cat #: T3DL-100, T3DL-200, T3DL-OEM, B-RL2

Description:

T3 DNA Ligase joins blunt end and cohesive end termini as well as repairs single-stranded nicks in duplex DNA. In the absence of 20-30% PEG 6000, T3 DNA Ligase displays a very low efficiency for blunt-ended ligation. T3 DNA Ligase displays a higher efficiency for joining A/T overhangs than C/G matched ends. T3 DNA Ligase retains 95% of its activity in 1.0 M NaCl or KCl, with an optimal concentration of 300 mM.

Protocol:

Combine 50 ng of vector with a 3-fold molar excess of insert. Adjust volume to 10 µl with dH_2O.
Add 10 µl of 2x Ligase Reaction Buffer and mix.
Add 1 µl of T3 DNA Ligase and mix thoroughly.
Centrifuge briefly and incubate at room temperature (25°C) for 15-30 minutes.
Chill on ice, then transform or store at -20°C.

Do not heat inactivate. Heat inactivation dramatically reduces transformation efficiency.