Ligation Protocol with T4 DNA Ligase (M0202) Version 2

New England Biolabs

Abstract

Please see the NEB website for more information.

Citation: New England Biolabs Ligation Protocol with T4 DNA Ligase (M0202). **protocols.io** dx.doi.org/10.17504/protocols.io.irscd6e
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Materials

T4 DNA Ligase M0202 by New England Biolabs

Protocol

Step 1.

Thaw the T4 DNA Ligase Buffer and resuspended at room temperature.

REAGENTS

T4 DNA Ligase Reaction Buffer - 6.0 ml B0202S by New England Biolabs

NOTES

Francisco Maresca 26 Sep 2015
A useful thing to do is to alicuote the 10x buffer less concentrated so when thawing the DTT gets soluble more easily.

Step 2.

Set up the reaction in a microcentrifuge tube on ice. (T4 DNA Ligase should be added last.)

COMPONENT	20 µl REACTION
T4 DNA Ligase Buffer (10X)*	2 µl
Vector DNA (4 kb)	50 ng (0.020 pmol)
Insert DNA (1 kb)	37.5 ng (0.060 pmol)
Nuclease-free water	to 20 µl
T4 DNA Ligase	1 µl

PROTOCOL

. T4 DNA Ligase Reaction

CONTACT: New England Biolabs

NOTES
New England Biolabs 14 Jul 2017

T4 DNA Ligase should be added last.

Note that the table shows a ligation using a molar ratio of 1:3 vector to insert for the indicated DNA sizes.

Use NEBioCalculator to calculate molar ratios.

ANNOTATIONS
Antonella Bastone 15 May 2018

Can all the quantities be proportionally scaled up for a final reaction volume of 300 µL? are there any considerations to take into account?

Step 2.1.
Vector DNA (4 kb) 50 ng (0.020 pmol)

AMOUNT
50 ng Additional info:

NOTES
Ben Claywell 17 Jul 2015
Use NEBioCalculator to determine concentration
Low Sin Yee 23 Jul 2015
recommended vector concentration 50ng=0.05ug
my linearized vector concentration= 5ug/ml=0.005ug/ul
1ul vector=0.005ug
how many ul of vector to make up 0.05ug?
(0.05ugx1ul)/0.005ug=10ul
thus, 10ul of vector should be added into ligation reaction.

Step 2.2.
Insert DNA (1 kb) 37.5 ng (0.060 pmol)

AMOUNT
38 ng Additional info:

NOTES
Ben Claywell 17 Jul 2015
Use NEBioCalculator to determine concentration

Step 2.3.
Nuclease-free water to 20 µl

Step 2.4.
T4 DNA Ligase, 1 µl

AMOUNT
1 µl Additional info:

REAGENTS
T4 DNA Ligase - 20,000 units M0202S by New England Biolabs

NOTES
mehrdad alirezaei 17 Jul 2015
T4 DNA Ligase - 100,000 units
Catalog #: M0202M

Step 2.5.

2 µl of T4 DNA Ligase Buffer (10X)

AMOUNT
2 µl Additional info:

REAGENTS
T4 DNA Ligase M0202 by New England Biolabs

Step 3.
Gently mix the reaction by pipetting up and down and microfuge briefly.

Step 4.
For cohesive (sticky) ends, incubate at 16°C overnight or room temperature for 10 minutes. For blunt ends or single base overhangs, incubate at 16°C overnight or room temperature for 2 hours.

NOTES
New England Biolabs 23 Sep 2014
Alternatively, high concentration of T4 DNA Ligase can be used in a 10-minute ligation for blunt ends.
Ben Claywell 17 Jul 2015
We are using sticky ends, so incubate at room temperature for 10 minutes.

Step 5.

Heat inactivate at 65°C for 10 minutes.

DURATION
00:10:00

NOTES
Maohan Su 07 Dec 2016

Why? No heat inactivation will interference with transformation?

ANNOTATIONS
Abhilek Nautiyal 30 Mar 2018

Why? No heat inactivation will interference with transformation?

Step 6.
Chill on ice and transform 1-5 µl of the reaction into 50 µl competent cells.

NOTES
Ben Claywell 17 Jul 2015

Use 25 uL DH5α cells, and add 2 uL of reaction mixture.