Quick Ligation Protocol (M2200) Version 2

New England Biolabs

Abstract

This protocols is to be performed with the Quick Ligation Reaction Buffer. Please see the NEB website for more information.

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Materials

Quick Ligation Kit - 30 rxns M2200S by New England Biolabs

Protocol

Step 1.

Set up the following reaction in a microcentrifuge tube on ice. (Use NEBioCalculator to calculate molar ratios.)

COMPONENT	20 µl REACTION
Quick Ligase Reaction Buffer (2X)*	10 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
Quick Ligase	1 µl

**The Quick Ligase Reaction Buffer should be thawed and resuspended at room temperature.*

REAGENTS
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NOTES
Stefanie Buchholz 02 Jun 2016

7.5 Vektor; mTRPC1-VL-ØPore_pMax_IRES-GFP_lin; 3.73ng/µl

2.5 Insert; mTRPC4-Pore_LH47/LH48; 22ng/µl 1:10verd.

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Quick 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.

Step 2.

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

Step 3.

Incubate at room temperature (25°C) for 5 minutes.

Step 4.

Chill on ice and transform 1-5 µl of the reaction into 50 µl competent cells. Alternatively, Store at -20°C.

NOTES

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