LifeSct.com
Life Scientists' Service Center

Email: support@lifesct.com
Tel: 240-715-2985 Fax: 240-252-7376
14323 Woodcrest Dr., Rockville, MD 20853

LiClone™ Fast DNA Ligation Kit

Cat. #: M0031 Size: 1 ml/5 ml

Introduction

LiClone™ Fast DNA Ligation Kit takes only 5 minutes at room temperature to complete the ligations of the DNA sticky ends or blunt ends using this kit. This kit contains Quick T4 DNA Ligase and the optimized 2× Quick Ligation Reaction Buffer. The ligation efficiency of the quick ligase is equivalent to the ligation with T4 DNA Ligase for 1 hour. The products can be used directly for transformation experiments.

Package Information

Components	M0009
Quick T4 DNA Ligase (15 U/μl)	100 μl
2× Quick Ligation Reaction Buffer	5×200 μl

Storage

All materials should be stored at -20°C.

Precautions

1. This kit allows most of the ligation reactions to be done at 25°C for 5 minutes or less. The increase of reaction time does not increase the ligation efficiency. One hour after the quick ligation, the transformation efficiency will be significantly reduced; if the reaction is left overnight at 25°C, the transformation efficiency will drop to 75%.

2. The 2× Quick Ligation Reaction Buffer contains ATP. Thaw it on ice before use and mix thoroughly. It is recommended to aliquot during first use, to avoid repeated freezing and thawing which will affect DNA ligation efficiency.

3. Because the T4 DNA Ligase contains glycerol, it is more viscous and easy to hang on the wall. It is recommended to collect the liquid to the bottom of the tube by brief centrifuge before use. When pipetting, don't put the tip too deeply, to avoid loss.

4. If the quick ligation product is used for electroporation, the PEG in the buffer will affect the efficiency of electroporation. It is recommended to use DNA purification kit (Cat. #: M0036) to purify the ligation product first, then do the electroporation.

Protocol

1. Reaction system:

Components	Reaction Volume
Vector DNA	X μl (10-100 ng)
Insert DNA	Y μl
2× Quick Ligation Reaction Buffer	10 μl
Quick T4 DNA Ligase (15 U/ul)	1 μl
RNase-Free Water	To 20 μl

Note: the molar ration of Vector DNA to Insert DNA is usually 1:3-1:8. The appropriate molar ratio can be selected according to experimental conditions.

2. Mix gently and centrifuge briefly. Leave the reaction at 25°C for 5 minutes.

Note: The reaction time should not exceed 15 minutes, otherwise it will reduce the ligation efficiency.

3. Do not use heating deactivation. Centrifuge briefly and collect the solution from the tube wall to the tube bottom.

Note: Due to the PEG in the buffer, heat deactivation significantly reduces transformation efficiency.

4. After the reaction is done, the DNA ligation product can be stored at 0-4°C and do the transformation experiment later; the ligation product can also be stored at -20°C.

Note: For chemical transformation, the amount of ligation product should not exceed 10% of the competent cell volume.

5. Add 1-2 μl ligation product into 50 μl component cells.

Note: 1) For chemical transformation, the amount of ligation product should not exceed 10% of the competent cell volume.

2) If the quick ligation product is used for electroporation, the PEG in the buffer will affect the efficiency of electroporation. It is recommended to use DNA purification kit (Cat. #: M0036) to purify the ligation product first, then do the electroporation.

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