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Quick DNA Ligation Kit

Product Number: DLK92

Shipping and Storage

-20°C.

Components

Component	DLK92 20rxns	DLK92 100rxns
Quick T4 DNA Ligase(15 U/μl)	20μl	100μl
2×Quick Ligation Reaction Buffer	200μl	5×200μl

Description

The rapid ligation kit can complete the ligation reaction of DNA sticky or even ends after 5 minutes of reaction at room temperature (25℃). The kit contains Quick T4 DNA Ligase and 2×Quick Ligation Reaction Buffer optimized for fast and efficient DNA ligation. The efficiency of fast connection is equivalent to using T4 DNA Ligase for regular connection for 1 hour. The rapid connection product can be directly used in conventional bacterial transformation experiments.

Note

1. This reagent kit can enable most connection reactions to reach the reaction endpoint within 5 minutes or even shorter at 25 ℃, and increasing the reaction time will not enhance the reaction efficiency. After 1 hour of rapid connection reaction, the conversion efficiency will significantly decrease; If the rapid connection reaction is carried out overnight at 25 ℃, the conversion efficiency will decrease to 75%.
2. 2×Quick Ligation Reaction Buffer contains ATP, which should be pre melted on ice and thoroughly mixed. It is recommended to divide it into small tubes for initial use and freeze it to avoid repeated freezing and thawing affecting DNA connection efficiency. Due to the presence of glycerol in T4 DNA Ligase, which is viscous and prone to wall adhesion, it is recommended to briefly centrifuge the liquid before use
3. Collect the body to the bottom of the tube. When sampling, try not to let the nozzle penetrate too deep into the liquid surface to avoid sticking to the nozzle and causing damage.
4. If the rapid ligation product is used for electroporation, PEG in the rapid ligation reaction system will affect the electroporation efficiency. It is recommended to use a centrifugal column to purify the ligation product with DNA (such as DNK2301 Pool DNA Mini Kit) before electroporation.

Protocol

1. Prepare the reaction solution according to the following system:

Reagent	20μLReaction system
Vector DNA	X μl(10-100 ng)
Insert DNA	Y μl
2×Quick Ligation Reaction Buffer	10 μl
Quick T4 DNA Ligase(15U/μl)	1 μl
RNase-Free Water	Up to 20 μl

Note: The usage of Insert DNA: The molar ratio of Vector DNA to Insert DNA is generally 1:3-1:8, and the appropriate molar ratio of Vector DNA to Insert DNA can be selected according to the experimental situation. The calculation method for DNA moles: DNA moles (nmol)=DNA mass (ng)/(660 daltons x number of inserted DNA bases bp).

2. Gently mix and briefly centrifuge. React at 25 ℃ for 5 minutes.

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

3. Do not carry out heating deactivation reaction. Instantly centrifuge and collect the solution on the tube wall to the bottom of the tube.

   Note: Due to the presence of PEG in the buffer, heating deactivation will significantly reduce conversion efficiency.

4. After the reaction is complete, store the DNA ligation product at 0-4℃ and then conduct a transformation experiment; The DNA ligation product can also be stored at -20℃.

   Note: When using chemical conversion, the amount of the connecting product should not exceed 10% of the volume of the competent cells.

5. Heat shock the connecting product to convert 50μl of competent cells, or take 1-2μl of the connecting product and electrocute it to convert 50μl of competent cells.

   Note: 1) When using chemical conversion, the amount of connecting product added should not exceed 10% of the volume of competent cells.

   2) If the rapid ligation product is used for electroporation, as PEG in the rapid ligation reaction system can affect the electroporation efficiency, it is recommended to use a centrifugal column to purify the ligation product with DNA (DNK2301 Pool DNA Mini Kit) before electroporation.