Speedy Ligase

Catalogue number: MB13001, 50 ligations

Description

Speedy Ligase is an improved DNA Ligase developed to carry out fast (less than 15 minutes) and efficient ligation of sticky or blunt-end DNA at room temperature. The enzyme catalyses the formation of a phosphodiester bond between juxtaposed 5'-phosphoryl and 3'-hydroxyl termini in duplex DNA. Rapid ligation is based on the combination of Speedy Ligase with a 4× Speedy ligation buffer.

Storage temperature

Speedy Ligase should be stored at -20 °C in a constant temperature freezer. The protein will remain stable till the expiry date if stored as specified.

Unit definition

One unit catalyses the exchange of 1 nmol of radiolabelled phosphate from pyrophosphate into Norit-absorbable material in 20 min at 37 °C under standard assay conditions.

Enzyme concentration: 10 U/µL

Inactivation

Speedy Ligase is heat inactivated at 65 °C for 10 min.

System components

Speedy Ligase is provided with a dedicated highly optimized NZYtech reaction buffer, which contains ATP that is critical for this enzyme. Repeated freeze-thaw cycles will affect the stability of ATP, so we recommend making 10-20 µL aliquots of the reaction buffer and store at -20 °C. Vortex the reaction buffer solution thoroughly after thawing and prior to use.

Speedy ligation protocol

The enzyme performs well at temperatures ranging from 16 °C to 25 °C. The optimal temperature for a ligation reaction is a balance between the enzyme's optimal temperature and the temperature required to ensure annealing of the DNA fragment ends, which can vary with the length and base composition of the terminal sequences.

We recommend using a 1:3-10 molar ratio of vector:insert. To calculate optimal amounts of insert DNA in ligation reaction, see below:

$$\frac{\text{ng of vector} \times \text{kb size of insert}}{\text{kb size of vector}} \times \text{molar ratio of } \frac{\text{insert}}{\text{vector}} = \text{ng of insert}$$

Example: If using 50 ng of a vector plasmid with 3 kb, for a 1:10 molar ratio of vector:insert then you will require the following amount of a 500 bp insert:

$$\frac{50 \times 0.5}{3} \times 10 = 83 \text{ ng}$$

1. On ice, in a sterile, nuclease-free microcentrifuge tube, prepare a reaction mixture, combining the following components (for a 20 µL reaction):

Component	Volume
4× Speedy ligation buffer (provided)	5 µL
Vector DNA (20-50 ng)	x µL
Insert DNA (3-10 molar excess)	y µL
Speedy Ligase (10 U/µL)	1 µL
Nuclease-free water	up to 20 µL

2. Mix and centrifuge briefly to bring the contents to the bottom of the tube.

3. Incubate at room temperature for 5-15 minutes (5 min for cohesive ends or 15 min for blunt ends). Longer incubation periods may lead to slightly higher ligation efficiency.

4. Use the ligation reaction to transform NZYtech competent cells.

Important notes

- 4× Speedy ligation buffer is highly viscous so it is recommended special care while pipetting.4× Speedy ligation buffer should be thoroughly vortexed before pipetting.
- Avoid multiple freeze thawing cycles with both enzyme and buffer.
- It is extremely important not to change the ratio of Speedy Ligase volume: final volume to prevent decrease in efficiency of cloning reactions.
- For blunt-end ligations, use higher quantities of both vector and insert DNA.
- For sticky (cohesive)-end ligations, we recommend to heat both vector and insert DNA prior to the ligation. If the ligation mixture will be used for electroporation, a DNA purification step is recommended before the transformation. Use a spin column purification method (NZYGelpure, NZYtech, Cat. No. MB011) or chloroform extraction.

Quality control assays

Purity

Recombinant Speedy Ligase is >95% pure as judged by SDS polyacrylamide gel electrophoresis followed by Coomassie blue staining.

Nuclease assays

0.2-0.3 µg of pNZY28 plasmid DNA are incubated with 10 U of Speedy Ligase in 1× Speedy ligation buffer for 14-16 hours at 37 °C. Following incubation, the DNA is visualized on a GreenSafe-stained agarose gel. There must be no visible nicking or cutting of the DNA.

Functional assay

Linearized pNZY28 plasmid (leaving either blunt-end or cohesive ends) is re-ligated with 5 units of Speedy Ligase. The DNA is then transformed into NZY5α competent cells that are plated on ampicillin plates. The re-ligation efficiency is determined by counting transformed bacterial colonies.

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