BIONEER

AccuPower® Ligation PreMix

ISO 9001 Certified

The application number : 10-2007-0004766

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***AccuPower*® Ligation PreMix is a New, Powerful, Ready-to-use Ligation reagent optimized for more accurate Ligation reactions.**

Description

T4 DNA Ligase catalyzes the formation of a phosphodiester bond between juxtaposed 5' phosphate and 3' hydroxyl termini in duplex DNA or RNA. This enzyme combines blunt-end and cohesive-end termini as well as repairs single-stranded nicks in duplex DNA, RNA, or DNA/RNA hybrids.

Source

T4 DNA Ligase was isolated from a recombinant *E. coli* strain.

Storage Condition

4℃ to -20℃ (-20℃ is recommended for longer storage.)

Applications

- Gene cloning: Joining double-stranded DNAs with cohesive or blunt ends.
- Cloning into vectors
- Library construction
- TA cloning
- Linker ligation
- Recirclization of linear DNA

Advantages

Speed

Only 5 minutes for ligation of cohesive end DNA and 10 minutes for ligation of blunt end DNA at room temperature

Stability

Stabilized for 4 months at room temperature and 3 years at – 20℃

Simplicity

Ready to use, *AccuPower*® Ligation PreMix is a single-tube lyophilized premix including the necessary components for ligation reaction such as T4 DNA Ligase, ATP, reaction buffer and stabilizer.
You can finish 96 samples ligation within 30 minutes by only adding your vector and insert DNA.

Reproducibility and Yield

Strict functional QC assays provide highly consistent and reproducible ligation performance. In most applications, an increase in yield is observed as compared to standard reactions.

Unit Definition

2 Weiss units / Reaction. One Weiss[3] unit is defined as the amount of enzyme required to catalyze the conversion of 1 nmole of ^{32}P pyrophosphate into Norit-adsorbable material in 20 minutes at 37℃. 0.01 Weiss unit of T4 DNA Ligase is the amount of enzyme required to catalyze ligation reaction of greater than 95 % of 1 ㎍ of Lambda DNA / *Hin*d III fragment in 20 minutes at 16℃.

Quality Controls

- Cohesive-end Ligation: > 95% of 1 ㎍ Lambda / *Hin*d III fragments are ligated within 5 minutes in 20 ㎕ reaction condition.
- Blunt-end Ligation: > 95% of 1 ㎍ Lambda / *Eco*R V fragments are ligated within 10 minutes in 20 ㎕ reaction condition.

Experimental Protocol

1. Prepare mixture of insert DNA and vector using the molar ratio specified by your standard ligation protocol.
   (We recommend the molar ratio of vector : insert DNA = 1 : 3)

   Molar ratio: $\frac{\square \text{ ng of vector} \times \square \text{ kb size of insert DNA}}{\square \text{ kb size of vector}} \times 3 / 1^{a} = \square \text{ ng of insert DNA}$

   [a] : molar ratio of insert DNA / vector
2. Add 20 ㎕ of the mixture to *AccuPower*® Ligation PreMix tube. If the volume of mixture is less than 20 ㎕, add distilled to water to adjust the final volume to 20 ㎕.
3. Mix completely by pipetting and vortexing vigorously and then spin down briefly.
4. Incubate the reaction mixture at room temperature for 5~10 minutes.
5. Collect 10 ㎕ from tube and perform transformation with 100 ㎕ of competent cells.
   In case an electroporation of ligated products, salt precipitate the DNA before electroporation in order to remove the salts contained in ligation mixture.

Heat Inactivation

70℃ for 10 minutes.

References

1. Engler, M. J. and Richardson, C. C. (1982) In: *The Enzymes*, Boyer, P. D. ed., Academic Press, New York, NY.
2. Zimmerman, S. B. and Pheiffer, B. H. (1983) *Proc. Natl. Acad. Sci.* USA 80, 5852-5856
3. Weiss, B., et al.,(1968) *J. Biol. Chem*., 243, 4543-4555

Ordering Information

Cat. No.	Description
E-3061	T4 DNA Ligase (20,000 unit)
E-3062	T4 DNA Ligase (100,000 unit)
E-3111	Thermostabe *Thermus filiformis*(*Tfi*) DNA Ligase (2,000 unit)
E-3112	Thermostabe *Thermus filiformis*(*Tfi*) DNA Ligase (10,000 unit)
K-7101	*AccuRapid*® DNA Ligation Kit (50 ligations)
K-7102	*AccuRapid*® DNA Ligation Kit (150 ligations)
K-7103	***AccuPower*® Ligation PreMix (96 reactions)**

A complete product list appears on our web site at *www.bioneer.com*.

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