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Simplifying Genomics

NxGen® T4 DNA Ligase High Concentration Rapid Kit

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Technical Support

Lucigen is dedicated to the success and satisfaction of our customers. Our products are tested to assure they perform as specified when used according to our recommendations. It is imperative that the reagents supplied by the user are of the highest quality. Please follow the instructions carefully and contact our technical service representatives if additional information is necessary. We encourage you to contact us with your comments regarding the performance of our products in your applications. Thank you.

Lucigen Technical Support
Email: techserv@lucigen.com
Phone: (888) 575-9695

Product Guarantee: Lucigen guarantees that this product will perform as specified for one year from the date of shipment. Please avoid using reagents for greater than one year from receipt.

Product Description

T4 DNA Ligase catalyzes the formation of a phosphodiester bond between the terminal 5′ phosphate and a 3′ hydroxyl groups of duplex DNA or RNA. The enzyme efficiently joins blunt and cohesive ends and repairs single stranded nicks in duplex DNA, RNA, or DNA/RNA hybrids (1).

Storage buffer: T4 DNA Ligase is supplied in**:** 10 mM Tris-HCl, 50 mM KCl, 1 mM dithiothreitol, 0.1 mM EDTA, 0.1%Triton X-100, 50% glycerol, pH 7.5 @ 25 °C.

2X Rapid Ligation Buffer is composed of 132 mM Tris-HCl, 20 mM MgCl2, 2 mM dithiothreitol, 2 mM ATP, 15% PEG, pH 7.6 @ 25 °C.

10X T4 DNA Ligase Buffer is composed of 500 mM Tris-HCl, 100 mM MgCl2, 50 mM dithiothreitol, 10 mM ATP, pH 7.6 @ 25 °C.

Source of protein: A recombinant *E. coli* strain carrying the cloned T4 DNA Ligase gene.

Unit Definition: One Weiss unit is defined as the amount of enzyme required to convert 1 nmol of ^{32}P-labeled inorganic pyrophosphate into Norit absorbable material in 20 minutes at 37°C, using specified reaction conditions(2).

Note: 1 Weiss Unit is approximately 67 cohesive end units.

Product Specifications

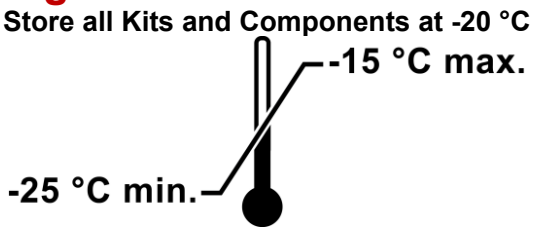
TEST	SPECIFICATION
Purity (SDS-PAGE)	>99%
SS Exonuclease	6,000 U <0.1% released
DS Exonuclease	6,000 U <0.1% released
Endonuclease	6,000 U <0.1% converted
E. coli 16S rDNA Contamination	3,000 U <10 copies

Product Designations and Kit Components

Product	Ligase Concentration	Kit Size	Catalog Number	Reagent Description	Part Numbers	Volume
NxGen® T4 DNA Ligase (High Concentration)	10 Units* /µL	1,500 Units*	30243-1	T4 DNA Ligase (High Concentration)	F83914-1	150 µL
				10X T4 DNA Ligase Buffer	F88912-1	1.5 mL
				2X Rapid Ligation Buffer	F88915-1	1.5 mL
		7,500 Units*	30243-2	T4 DNA Ligase (High Concentration)	F83914-1	5 x 150 µL
				10X T4 DNA Ligase Buffer	F88912-1	5 x 1.5 mL
				2X Rapid Ligation Buffer	F88915-1	5 x 1.5 mL

*Weiss Units

Components & Storage Conditions



Rapid Ligation Reaction Set-Up

1. Add all of the components below to a clean reaction vessel.

Quantity	Component
10 µL	2X Rapid Ligase Buffer
X	Vector (1-10 ng/µL)
Y	Insert (1-10 ng/µL)
1 µL	T4 DNA Ligase (10 U/µL)
To 20 µL	Nuclease-free Water

2. Mix well by pipetting.
3. Incubate at 25 °C for 10 minutes
4. Purify DNA using a PCR clean-up column and elute in ~50 µL.

   – OR –

   Immediately dilute in TE or water (at least 1:10).

   Note: Heat inactivation of ligation reactions containing 2X Rapid Ligation Buffer is not recommended, as it may reduce transformation efficiency.

5. Transform 0.1-10 ng of ligation product into a chemically or electrocompetent cell line that is compatible with the vector.

Notes:

- High-concentration T4 DNA Ligase in combination with the 2X Rapid Ligation buffer greatly stimulates the rate and efficiency of blunt-end ligation; therefore, incubations of over 10 minutes are NOT recommended and can greatly reduce the transformation efficiency. Use the recommended protocol to maximize transformation efficiency.

- 10X T4 DNA Ligase Buffer does not contain PEG and is compatible with Standard Ligation Reaction (below), includes longer incubation and heat inactivation.

Standard Ligation Reaction Set-Up

1. Add all of the components below to a clean reaction vessel.

Quantity	Component
2 µL	10X T4 Ligase Buffer
X	Vector (1-10 ng/µL)
Y	Insert (1-10 ng/µL)
1 µL	T4 DNA Ligase (10 U/µL)
To 20 µL	Nuclease-free Water

2. Mix well by pipetting.
3. Incubate at 25 °C for 30 minutes.
4. Heat inactivate the reaction by incubating the ligation at 70 °C for 15 minutes.

5. Purify DNA using a PCR clean-up column and elute in ~50 µL.

   – OR –

   Immediately dilute in TE or water (at least 1:10).

6. Transform 0.1-10 ng of the ligation product into a chemically competent or electrocompetent cell line that is compatible with the vector.

References

1. Engler, M. J., and Richardson, C. C. (1982) DNA ligases. In *The Enzymes*, Vol. XV (Ed. P. D. Boyer) Academic Press, New York, 3-29.

2. Weiss, B., Thompson, A., and Richardson, C. C. (1968) Enzymatic breakage and joining of deoxyribonucleic acid, VII. Properties of the enzyme-adenylate intermediate in the polynucleotide ligase reaction. J. *Biol. Chem*. 243, 4556-4563.

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