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Ampligase® Thermostable DNA Ligase

Cat. Nos. A8101, A30201, A0110K, A3210K, A0102K, A32250, A32750, A3202K, A1905B, and A3201S

Ampligase® Thermostable DNA Ligase catalyzes the NAD-dependent ligation of adjacent 3′-hydroxyl and 5′-phosphate termini in duplex DNA structures. Derived from a thermophilic bacterium, Ampligase DNA Ligase is stable and active at much higher temperatures than conventional DNA ligases. The half-life of Ampligase DNA Ligase is 48 hours at 65°C and more than 1 hour at 95°C. In most cases, the upper limit on reaction temperatures with Ampligase DNA Ligase is determined by the T_m of the DNA substrate. Under conditions of maximal hybridization stringency, nonspecific ligation is nearly eliminated. Ampligase DNA Ligase has no detectable activity on blunt ends or RNA substrates. The enzyme is active in a variety of DNA polymerase buffers within a pH range of 7-8. Ampligase DNA Ligase does not replace T4 DNA Ligase in most conventional cloning applications because it has: i) no activity on blunt ends; and ii) low activity at temperatures where 2- and 4-base cohesive ends form stable duplexes.

Cat. #	Concentration	Quantity
Ampligase DNA Ligase Kits contain: Ampligase DNA Ligase, 10X Reaction Buffer, and Control DNA.		
A8101	@ 5 U/µl	1,000 U
A30201	@ 5 U/µl	5,000 U
Ampligase Enzyme & Buffer contain: Ampligase DNA Ligase and 10X Reaction Buffer (25 µl/50 U enzyme).		
A0102K	@ 100 U/µl	2,500 U
A32250	@ 5 U/µl	250 U
A32750	@ 5 U/µl	750 U
A3202K	@ 5 U/µl	2,500 U
Ampligase DNA Ligase (enzyme only).		
A0110K	@ 100 U/µl	10,000 U
A3210K	@ 5 U/µl	10,000 U
Ampligase 10X Reaction Buffer (buffer only).		
A1905B		5 ml
Ampligase 1X Storage Buffer (buffer only).		
A3201S		1 ml

Ampligase Thermostable DNA Ligase is the ideal enzyme for applications that require high-temperature, high-stringency ligation of duplex DNA structures, including:*

– Ligation Amplification[1-11]
– Repeat Expansion Detection[12-14]
– High-Fidelity Gene Synthesis from overlapping oligodeoxynucleotides[15,16]
– Multiple Site Mutagenesis[17]
– Targeted Inverted Repeat Amplification[18]

Product Specifications

Storage: Store only at –20°C in a freezer without a defrost cycle.

Storage Buffer: Ampligase Thermostable DNA Ligase is supplied in a 50% glycerol solution containing 50 mM Tris-HCl (pH 7.5), 100 mM NaCl, 0.1 mM EDTA, 0.1% Triton® X-100, and 1 mM dithiothreitol.

Reaction Buffer: 10X Ampligase Reaction Buffer contains 200 mM Tris-HCl (pH 8.3), 250 mM KCl, 100 mM $MgCl_2$, 5 mM NAD, and 0.1% Triton® X-100.

Unit Definition: One unit of Ampligase Thermostable DNA Ligase catalyzes the ligation of 50% of the *cos* sites present in 1 µg of bacteriophage lambda DNA in 1 minute at 45°C.

Note: *One Ampligase unit is equivalent to at least 15 "cohesive end units" or the "nick closing units" of thermostable ligase described elsewhere.*

Stability: Enzyme activity was unchanged after incubation in 1X Ampligase Reaction Buffer for 1 week at 37°C. The half-life of the enzyme in 1X Reaction Buffer is ~48 hours at 65°C and >1 hour at 95°C.

Quality Control: Ampligase DNA Ligase activity is assayed in a reaction containing 1 µg of bacteriophage lambda DNA digested with *Sal* I and *Sma* I (Control DNA), 1X Ampligase Reaction Buffer, and varying amounts of enzyme for 100 minutes at 45°C. Results are assayed by agarose gel electrophoresis (see page 3).

Contaminating Activity Assays: Ampligase DNA Ligase is free of detectable exo- and endonuclease and RNase activities as judged by gel electrophoresis following incubation of 1 µg of DNA and RNA substrates with 100 U of Ampligase enzyme for 16 hours at 70°C.

Related Products: The following products are also available:

– Fast-Link™ DNA Ligation Kits
– Fast-Link™ DNA Ligation and Screening Kit
– Colony Fast-Screen™ Kit
– T4 DNA Ligase
– T4 Polynucleotide Kinase
– Transformation and Storage Solution
– TransforMax™ Electrocompetent *E. coli*
– End-It™ DNA End-Repair Kit

Applications of Ampligase Thermostable DNA Ligase

Ligation Amplification[1-11] also called the nick ligation assay or ligation chain reaction (LCR), is a simple and sensitive technique that permits the user to detect any defined DNA sequence by geometric amplification of ligation products complementary to the target sequence. Ligation amplification provides the basis for simple "+/–" assays with the power to distinguish DNA sequences that differ by as little as a single base. For applications requiring detection and discrimination of specific sequences (e.g., genetic screening, allelic classification), ligation amplification has advantages over polymerase chain reaction (PCR) with respect to specificity, sensitivity, and simplicity. Because the method can detect attomolar (10^{-18}) amounts of target DNA, ligation amplification also offers an extremely sensitive assay for detecting viral or bacterial infections and somatic mutations.

Repeat Expansion Detection[12-14] (RED) is a ligation-based method of genetic screening that detects DNA regions comprised of multiple trinucleotide repeats. Some regions of repeated trinucleotides in humans are genetically unstable and prone to duplication and expansion. If the resulting "expanded repeat" occurs within a gene, it may lead to genetic disease. Known examples of human diseases which can be caused by expanded repeats include myotonic dystrophy, spinobulbar muscular atrophy, Huntington's Disease, and fragile-X syndrome. In RED, the genomic DNA of interest is hybridized to an oligonucleotide probe that contains multiple copies of a trinucleotide. If the trinucleotide is present in the target DNA in expanded repeats, two or more of the oligo probes will hybridize in adjacent positions. Ampligase DNA Ligase then ligates the adjacent oligo probes, forming multimeric ligation products. The denaturation/annealing/ligation cycle is repeated up to 500 times in a thermal cycler to amplify the signal.

High-Fidelity Gene Synthesis[15,16] from oligonucleotides that contain long regions of complementary overlap, is improved with Ampligase DNA Ligase. Although genes have been synthesized using T4 DNA Ligase, the probability of forming the correct ligation products is greatly increased by using higher ligation temperatures. Sutton *et al.* used Ampligase DNA Ligase and oligos from 29-78 bp in length to synthesize a modified form of the 1,800-bp *cry*IIIA gene from *Bacillus thuringiensis*. Previous attempts using T4 DNA ligase had yielded a high percentage of incorrectly ligated molecules.

Simultaneous Multiple-Site Mutagenesis[17] is a novel ligation-based method for the simultaneous, site-specific mutagenesis of multiple loci. The method utilizes Ampligase DNA Ligase to catalyze the ordered ligation of amplified DNA fragments that have had point mutations introduced via mutant primers.

Targeted Inverted Repeat Amplification[18] is a method for characterizing unknown regions of flanking DNA involves generating an intramolecular stem-loop structure that contains the flanking DNA in a restriction fragment adjacent to the known sequence. When hybridized to a suitable oligonucleotide, this stem-loop structure serves as a substrate for Ampligase DNA Ligase. The product of this reaction can readily be amplified, sequenced and further characterized. Because the method does not require any cloning, it can be used to characterize regions of flanking DNA that are refractory to cloning. Applications include chromosome walking, characterizing unclonable DNA, defining intron/exon boundaries, characterizing viral and transposon insertion sites, and generating yeast artificial chromosomes.

Ampligase Control Reaction

Control Reaction: The Ampligase Ligation Control DNA included in the Ampligase Kits (Cat. # A00101 and A30201) is bacteriophage lambda DNA that has been digested with the restriction enzymes *Sma* I and *Sal* I. Treatment of the Ligation Control DNA with Ampligase DNA Ligase demonstrates the enzyme's activity on nicked duplex DNA structures and its lack of activity on blunt-ended DNA.

Digestion of lambda DNA with *Sma* I and *Sal* I generates four major fragments. Fragments 1 and 3 have a *cos* site at one end and a cleaved restriction site at the other end. Fragments 2 and 4 have cleaved restriction sites at both ends. After incubating the Control DNA at 45°C with Ampligase Ligase, fragments 1 and 3 anneal at the *cos* site, a 12-base overlap that is stable at 45°C. Ampligase Ligase then covalently closes the nicks at either end of the annealed *cos* site, forming a larger fragment "1 + 3". Fragments 2 and 4 are not affected for two reasons: i) the enzyme has no activity on the *Sma* I blunt ends; and 2) the four-base *Sal* I cohesive ends do not anneal at 45°C and thus cannot be ligated.

Control Reaction Protocol:

1. Label one tube "Control DNA Only" and another tube "Control DNA + Ampligase Enzyme".
2. Add to each tube:

41 µl	sterile deionized water
5 µl	10X Ampligase Reaction Buffer
4 µl	Ampligase Ligation Control DNA
50 µl	Total volume

3. Add 1 unit of Ampligase enzyme to the tube marked "Control DNA + Ampligase enzyme".
4. Incubate both tubes for 10 minutes at 45°C.
5. Add 10 µl of a Stop/Gel Loading Solution (e.g., 40% [w/v] sucrose, 0.1 M EDTA [pH 7.0], 0.25% bromophenol blue, and 1% SDS) to each tube and mix.
6. Heat both tubes at 70°C for 10 minutes to denature unligated *cos* sites.
7. Load 3 µl of each reaction into adjacent lanes on a 0.8% agarose gel.
8. Run the gel slowly (overnight) at 30 volts to allow resolution of bands in excess of 20 kb.
9. Stain and visualize the gel after electrophoresis.

***Note:** Some applications in which this product may be used may be covered by patents or patent applications applicable in certain countries. Because purchase of this product does not include a license to perform any patented application, users of this product may be required to obtain a license depending upon the particular application and country in which the product is used.*

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