ALPHA DIAGNOSTIC INTERNATIONAL

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Product Data Sheet

Cat # RP-374	Recombinant T4 DNA Ligase	**Size**: 20KU

Source: Escherichia Colilambda lysogen NM 989 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 will join blunt end and cohesive end termini as well as repair single stranded nicks in duplex DNA, RNA or DNA/RNA hybrids. 50mM Tris-HCl (pH 7.8 at 25^{O}C), 10mM $MgCl_2$, 10mM DTT, 1mM ATP, 25 µg/ml BSA and DNA (0.1 to 1 µM in 5′ termini). Optimal ligation occurs at 16^{o}C.

Applications and Suggested Dilutions: Buffer: 50mM KCl, 10mM Tris-HCl (pH 7.4), 0.1mM EDTA, 1mM DTT, 200 µg/ml BSA and 50% glycerol. Store at -20^{o}C. Cloning of restriction fragments. Joining linkers and adapters to blunt-ended DNA Users must optimize the appropriate concentration and conditions for each assay.

Storage and Stability: Two years when stored at –20°C, 2 weeks at 4°C. If supplied in powder then reconstitute it in 100 ul water for 1 mg/ml stock and store in liquid at 4oC for ~1 week or aliquots in suitable size and store at -20oC for long term storage.

Unit Definition: 1. One unit is defined as the amount of enzyme required to give 50% ligation of Hind III fragments of λ DNA (5′ DNA termini concentration of 0.12 µM, 300- µg/ml) in a total reaction volume of 20 µl in 30 minutes at 16°C in 1X T4 DNA Ligase Reaction Buffer. 2.One Weiss unit is defined as the amount of enzyme required to catalyze the exchange of 1 nmol of ^{32}P from pyrophosphate to ATP, into Norit-adsorbable material in 20 minutes at 37°C.

Biological Activity: One Weiss unit is equivalent to circa 67 cohesive-end ligation units. T4 DNA Ligase is strongly inhibited by NaCl or KCl if the concentration is > 200mM. ·Ligation of blunt-ended and single-base pair overhang fragments requires about 50 times as much enzyme to achieve the same extent of ligation as cohesive-end DNA fragments. Blunt-end ligation may be enhanced by addition of PEG 4000 (10% w/v final concentration) or hexamine chloride, or by reducing the ATP concentration to 50µM. ·To dilute T4 DNA Ligase that will subsequently be stored at –20^{0}C, 50% glycerol storage buffer should be used; to dilute for immediate use, 1x T4 DNA Ligase reaction buffer can be used

Heat Inactivation: T4 DNA Ligase can be inactivated by incubation at 65^{o}C for 10 minutes.

Usage: This item is for LABORATORY RESEARCH USE ONLY. The product may not be used as drugs, agricultural or pesticidal products, food additives or household chemicals.

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