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DATA SHEET

> T4 DNA Ligase

T4 DNA Ligase (isolated and purified from an E. coli strain) catalyzes the formation of a phosphodiester bond between juxtaposed5'-phosphate and 3'-hydroxyl groups in duplex DNA or RNA. This enzyme is capable of joining adjacent nucleotides in either a blunt-ended or cohesive ended configuration, as well as repairing single-stranded nicks in duplex DNA, RNA or DNA/RNA hybrids.

cat. no.	*Weiss units*	*CE units*	*amount*	*note*
STS-TDL 100	400 Weiss	80000 units	160	10X T4 Ligase Buffer Standard or Fast
STS-TDL 1000	4x400 Weiss units	4x80000units	5x 160ul	10X T4 Ligase Buffer Standard or Fast

FOR RESEARCH USE ONLY

UNIT DEFINITION
One Weiss unit is defined as the amount of enzyme required to catalyze the exchange of 1 nmol of 32P from pyrophosphate to ATP, into Norit-adsorbable material in 20 minutes at 37 °C.

SHIPPING
Shipped in green ice.

STORAGE
Store at -20C°

SHELF LIFE
12 months. Avoid freeze/thaw cycles

CONCENTRATION

2.5 Weiss units/µl (500 CE units/µl)

FORM: liquid (Supplied in 10 mM Tris-HCl pH 7.4, 50 mM KCl, 0.1 mM EDTA, 1 mM DTT, 200 µg/ml BSA and 50 % [v/v] glycerol)

Standard Ligation Buffer, 10x conc.: 500 mM Tris-HCl pH 7.8 at 25 °C, 100 mM MgCl2, 100 mM DTT, 10 mM ATP and 25 µg/ml BSA

Fast Ligation Buffer, 2x conc.: 60 mM Tris-HCl pH 7.8 at 25 °C, 20 mM MgCl2, 20 mM DTT, 2 mM ATP and 10 % PEG

A white precipitate in the Ligation Buffer is normal and does not affect the reaction efficiency. Do not heat the buffer, as this will damage the contained ATP.

Heat inactivation:

T4 DNA Ligase can be inactivated by incubation at 65 °C for 10 minutes.

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Note:
• One Cohesive-End Ligation Unit (CEU) 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.
• One Weiss unit is equivalent to approx. 200 CE units.
• T4 DNA Ligase is strongly inhibited by NaCl or KCl if the concentration exceeds 200 mM.
• 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 50 µM.
• To dilute T4 DNA Ligase for subsequent storage at -20 °C a stor- age buffer containing 50 % glycerol should be used, to dilute Ligase for immediate use, 1x Reaction Buffer is recommended.

Assay Set-Up:

Standard Ligation Assay:

comp.	*final amount/conc.*	*20 **µl** assay*
Standard Ligation Buffer, 10x conc.	1X	2µl
Vector/Insert DNA	100 ng - 1 µg	100 ng - 1 µg
T4 DNA Ligase	0.1 - 1 Weiss units	0.04-0.4 µl
PCR-grade Water	-	fill up to 20 µl

Incubate for 20 - 30 min at 16 °C for optimal ligation.

Fast Ligation Assay

comp.	*final amount/conc.*	*20 **µl** assay*
Fast Ligation Buffer, 2x conc.	1X	10µl
Vector/Insert DNA	100 ng - 1 µg	100 ng - 1 µg
T4 DNA Ligase	0.1 - 1 Weiss units	0.04-0.4 µl
PCR-grade Water	-	fill up to 20 µl

Incubate for 5 min for cohesive-ended ligations or 15 min for blunt-ended ligations at ambient temperature (20 - 25 °C).