T4 DNA Ligase (5U/µl)

#GC03.1000

(for research only)

Product: T4 DNA Ligase is a 55.3kDA monomeric enzyme that catalyzes the formation of phosphodiester bonds between adjacent 5´- phosphate and 3´-hydroxyl termini in double-stranded DNA and/or double-stranded RNA. T4 DNA Ligase is capable of joining blunt and complementary cohesive ends, making it the Ligase of choice for restriction-ligation cloning of DNA fragments into plasmids. Moreover, this enzyme can be used for nick-repair as it closes nicks in double-stranded DNA or DNA/RNA hybrids.

Source: Purified from *E.coli* harbouring gene 30 from bacteriophage T4

Quantity: 1000U at a concentration of 5 Weiss Units/µl in storage buffer (20mM Tris-HCl (pH 7.5), 50mM KCl, 1mM DTT, 0.1mM EDTA, and 50% glycerol), supplied with 1,5ml of T4 DNA Ligation Buffer (5X) containing ATP.

Unit Definition: One Weiss unit is defined as the amount of enzyme required to catalyze the conversion of 1nmol of [^{32}PPi] into Norit®-absorbable form at 37ºC in 20 min, using specific reaction conditions (1). One Weiss units is the equivalent of approximately 200 CE units (Cohesive End ligation units), whereas one CEU is defined as the amount of enzyme required for 50% ligation of HindIII fragments of 1µg of lambda DNA at 16ºC in 30 min.

Properties: T4 DNA Ligase has an optimal reaction temperature of 22ºC but can be used in a wide temperature range (16ºC-37ºC). The enzyme can be heat inactivated at 65ºC for 10min, or chemically inactivated by NaCl or KCl at final concentrations above 200mM.

Appearance: Transparent liquid

QC: Enzyme activity is assayed by incubation of 3.3µM [^{32}PPi] in 66mM Tris-HCl (pH 7.6), 6.6mM $MgCl_2$, 0.066mM ATP, 10mM DTT, as well as by a blue/white colony assay. Absence of endonucleases and exonucleases is ascertained by appropriate assays.

Storage: -20ºC for at least 1 year.

1. Weiss, B., *et al*. (1968), Enzymatic breakage and joining of deoxyribonucleic acid, *J. Biol. Chem*., **243**, 4543-4555.

PROTOCOLS

It is recommended to, prior to use, thaw the T4 DNA Ligation Buffer (5X) at room temperature and vortex vigorously to mix components. The concentrated buffer might contain a white precipitate, which does not result in a loss of performance. Do not heat the five-times concentrated T4 DNA Ligation Buffer, to dissolve any precipitate, as it contains ATP, which is temperature-sensitive. It is also recommended to prepare aliquots (both T4 DNA Ligase and T4 DNA Ligation Buffer (5X)) to minimize contamination risks with nucleases. Before use, centrifuge the T4 DNA Ligase for a few seconds to ensure all liquid is at the bottom.

Cohesive Ends Ligation (sticky ends):

1. Add the following components in a DNase-free tube:
    - 10-20ng of pre-cut DNA vector
    - **x** ng pre-cut insert DNA (x= conform desired molar ratio insert:vector)
    - 2 µl T4 DNA Ligation Buffer (5X)
    - 1 µl T4 DNA Ligase (5U/µl)
    - nuclease-free water up to 10µl
2. Incubate at 22ºC for 5-15 min.
3. (Optional): heat-inactivate at 65ºC for 10min.
4. Use 5-10µl of the ligation mixture for the transformation of 50µl competent cells.

Blunt Ends Ligation:

1. Add the following components in a DNase-free tube:
    - 10-20ng of pre-cut, no religable DNA vector
    - **x** ng blunt insert DNA (x= conform desired molar ratio insert:vector)
    - 2 µl T4 DNA Ligation Buffer (5X)
    - 1 µl T4 DNA Ligase (5U/µl)
    - (Optional: 1µl PEG 6000***** (10x) [not provided])
    - nuclease-free water up to 10µl
2. Incubate at 22ºC for 5-15 min.
3. (Optional): heat-inactivate at 65ºC for 10min (do not perform if PEG 6000 was included!).
4. Use 5-10µl of the ligation mixture for the transformation of 50µl competent cells.

*******The addition of polyethylene glycol (PEG 6000), greatly increases the ligation efficiency of blunt-ended DNA ligation. If desired, one can inactivate T4 DNA Ligase, in the presence of PEG, by adding NaCl or KCl to a final concentration of above 200mM.*

Insert to Vector Molar Ratio:

For successful ligation, the “Insert-to-Vector” molar ratio should be in the range of 1:1 to 5:1, with higher ratios more commonly used (e.g. 3:1 for cohesive ends ligation and 5:1 for blunt ends ligation). Example: for a 5:1 ratio, the amount of insert (in ng) to be used should be:

ng insert = 5x amount of vector (ng) x (size insert (bp)/size vector (bp))

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