GeneBio Systems, Inc.™

GB-Clone™ T4 DNA Ligase

Description:

GB-Clone ™ T4 DNA Ligase catalyzes the formation of phosphodiester bonds between juxtaposed 5'phosphate and 3'hydroxyl termini in double-stranded DNA using ATP as a coenzyme. Both blunt and cohesive end DNA ligation, as well as single-stranded nick repair of DNA, RNA and DNA/RNA, are possible via the T4 DNA ligase. This enzyme is supplied in a buffer of 10 mM Tris-HCl (pH 7.5), 50 mM KCl, 10 mM 2-mercaptoethanol, 0.1 mM EDTA and 50 % glycerol.

Biological Source:

E. coli strain expressing a recombinant clone.

Molecular Weight:

68kDa.

Requirements:

Mg^{2+}, ATP and DTT. The optimum concentration of Mg^{2+} is 10mM. Mn^{2+} may be substituted for Mg^{2+} but is only 25% as effective as Mg^{2+}.

Inhibition:

50% inhibition by greater than 150mM NaCl (activity measured at nicks. Other inhibitors include 0.2M K^+, Cs^+, Li^+, NH_4^+ and 1mM spermine.

Inactivation:

Heat to 70°C for 10 minutes.

Contents

T4 DNA Ligase (200 U/μl)

10 X Ligation Buffe

Applications

- Cloning of restriction fragments.
- Joining linkers and adapters to blunt-ended DNA

Unit Definition

One unit of T4 DNA Ligase 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.

Storage: Store at -20°C

Storage Buffer

50 mM KCl, 10 mM Tris-HCl (pH 7.4), 0.1 mM EDTA, 1 mM DTT, 200 μg/ml BSA and 50% glycerol.

10x T4 Ligase Buffer:

660mM Tris-HCl(PH7.6), 66mM $MgCl_2$, 100mM DTT, 1mM ATP

Physical Purity

The purity is ≥90% as judged by SDS-polyacrylamide gel electrophoresis with Coomassie® blue staining.

Standard Applications

We recommend using a 1:1, 1:3 or 3:1 molar ratio of vector:insert DNA when cloning a fragment into a plasmid vector. These ratios will vary with other types of vectors, for example, cDNA and genomic cloning vectors. The following example illustrates the conversion of molar ratios to mass ratios for a 3.0kb plasmid and a 0.5kb insert DNA fragment.

Protocol:

The following ligation reaction of a 3kb vector and a 0.5kb insert DNA uses a 1:1 vector:insert ratio. Typical ligation reactions use 100–200ng of vector DNA.

1. Assemble the following reaction in a sterile microcentrifuge tube:

10 X Ligation Buffer	1 μl
vector DNA	100ng
insert DNA	1:1 to 10:1 molar ratio over vector
T4 DNA Ligase	0.5–1μl
Nuclease-Free Water	to final volume of 10μl

2. Incubate the reaction at room temperature for 3 hours, or 16°C for 4-18 hours, or 4 °C over night.

Notes:

1. Concatamers may form as ligation products. The extent of concatamer formation depends on the vector:insert ratio, incubation temperature and incubation time. This should be taken into account when screening transformants.

1-833 LabShop (1 -833 522 7467)
order@genebiosystems.com
www.genebiosystems.com

GeneBio Systems, Inc.™