SciPhi™ DNA Ligase.

Catalogue #NXG661-L/M/S

Catalogue No.	Product Components		
	SciPhi™ DNA Ligase (1000 CEU/µL)	SciPhi™ 10X DNA Ligase Buffer	SciPhi™ 50%PEG Solution
NXG661-L	40000 U	500 µl	300 µl
NXG661-M	20000 U	250 µl	150 µl
NXG661-S	10000 U	125 µl	75 µl
Store kit and all components at -20°C			

Product Description

SciPhi™ DNA Ligase catalyses the creation of a phosphodiester bond, linking adjacent 5'-phosphate and 3'-hydroxyl termini within double-stranded DNA or RNA. This enzyme is instrumental in the repair of single-strand nicks present in double-stranded DNA, RNA, or DNA/RNA hybrids. Moreover, it proficiently connects DNA fragments, whether they possess cohesive or blunt termini. Notably, the catalytic activity of DNA Ligase is dependent on ATP, serving as a crucial cofactor in these molecular processes

SciPhi™ Storage Buffer: The enzyme is supplied in: 50 mM KCl, 20 mM Tris-HCl (pH 7.5), 0.1 mM EDTA, 1 mM DTT and 50% (v/v) glycerol.

SciPhi™ 10X DNA Ligase Buffer: 400 mM Tris-HCl, 100 mM DTT, 100 mM MgCl2, 5 mM ATP (pH 7.8 at RT).

SciPhi™ 50% PEG Solution: 50% (w/v) polyethylene glycol 4000.

Source: *E.coli* cells with a cloned gene 30 from bacteriophage T4.

Molecular Weight: 55.3kDa.

Inhibition and Inactivation: DNA Ligase is inhibited by KCl or NaCl at high concentrations (>200 Mm). Inactivated by high temperature (65°C- 70°C) for 5-10 min.

Instruction for Use

DNA INSERT LIGATION INTO VECTOR DNA

Sticky-end ligation

1. Prepare the following reaction mixture:

Linear vector DNA	20-100 ng
Insert DNA	1:1 to 5:1(molar ratio over vector)
SciPhi™ 10X DNA Ligase Buffer	2 µl
SciPhi™ DNA Ligase	200 CEU
SciPhi™ Nuclease-free Water (NXG331)	Up to 20 µl
Total volume	20 µl

2. Incubate at 22°C for 10 min.
3. Utilize a maximum of 5 µl of the mixture for transforming 50 µl of chemically competent cells or 1-2 µl for every 50 µl of electrocompetent cells.

Note: The electro transformation efficiency may be improved by: heat inactivation of DNA ligase, purification of DNA or by chloroform extraction. Number of transformants increase by increasing the reaction time up to 1 hour.

Blunt-end ligation

1. Prepare the following reaction mixture:

Linear vector DNA	20-100 ng
Insert DNA	1:1 to 5:1(molar ratio over vector)
SciPhi™ 10X DNA Ligase Buffer	2 µl
SciPhi™ 50% PEG 4000 Solution	2 µl
SciPhi™ DNA Ligase	1000 CEU
SciPhi™ Nuclease-free Water (NXG331)	upto 20 µl
Total volume	20 µl

2. Incubate for 1 hour at 22°C.
3. Utilize a maximum of 5 µl of the mixture for transforming 50 µl of chemically competent cells. Purify DNA for electro-transformation, using the PCR Purification Kit or by chloroform extraction. Use 1-2 µl of DNA solution for every 50 µl of electrocompetent cells.

Note: If the ligation reaction mixture will be used for electroporation, replace the heat inactivation step with spin column purification or chloroform extraction.

SELF-CIRCULARIZATION OF LINEAR DNA

1. Prepare the following reaction mixture:

Linear DNA	10-50 ng
SciPhi™ 10X DNA Ligase Buffer	5 µl
SciPhi™ DNA Ligase	1000 CEU
SciPhi™ Nuclease-free Water (NXG331)	Up to 50 µl
Total volume	50 µl

2. Mix thoroughly followed by centrifuge for 1-2 min and incubate 10 min at 22°C.
3. Utilize a maximum of 5 µl of the mixture for transforming 50 µl of chemically competent cells or 1-2 µl for every 50 µl of electrocompetent cells.

Note: The electro transformation efficiency may be improved by: heat inactivation of DNA ligase, purification of DNA or by chloroform extraction. Number of transformants increase by increasing the reaction time up to 1 hour.

Suggestions & Recommendations

- The ligation efficiency of blunt-end DNA ligation is significantly enhanced by the presence of polyethylene glycol (PEG). It is recommended to use a concentration of 5% (w/v) PEG 4000 in the ligation reaction mixture.
- Do not use excessive amount of DNA Ligase in the rection mixture.
- The interaction between DNA Ligase and DNA can cause a mobility shift in agarose gels. To prevent this, treat samples with 6X Loading Dye & SDS Solution at 65°C for 10 min and then promptly cool on ice before loading.
- To achieve optimal transformation, the volume of the ligation reaction mixture does not surpass 10% of the competent cell volume.

LINKER LIGATION

Double-stranded oligonucleotide linkers are frequently employed to create overhangs that are absent in the insert. These linkers typically include recognition sequences for restriction enzymes, which are later digested post-ligation to produce overhangs compatible with cloning vectors. Alternatively, some linkers may possess overhangs ready for direct ligation with a cloning vector, eliminating the need for additional manipulation after ligation.

1. Prepare the following reaction mixture:

Linear DNA	100-500 ng
Phosphorylated linkers	1-2 µg
SciPhi™ 10X DNA Ligase Buffer	2 µl
SciPhi™ 50% PEG 4000 Solution	2 µl
SciPhi™ DNA Ligase	400 CEU
SciPhi™ Nuclease-free Water (NXG331)	Up to 20 µl
Total volume	20 µl

2. Mix thoroughly followed by brief centrifugation and incubate for 1 hour at 22°C.
3. Heat inactivates at 70°C for 5 min or at 65°C for 10 min.

Note: DNA Ligase exhibits activity in both PCR and restriction digestion buffers. Consequently, linker ligation reactions can be conducted in the optimal restriction enzyme buffer for subsequent digestion. In such cases, it is recommended to supplement the ligation reaction with ATP to a final concentration of 0.5 mM. After inactivation of DNA Ligase, introduce the restriction enzyme directly into the reaction mixture and incubate according to the digestion protocol.

Applications

- Cloning of PCR products.
- Cloning of restriction enzyme generated DNA fragments.
- Joining of double-stranded oligonucleotide linkers or adaptors to DNA.
- Amplified fragment length polymorphism (AFLP).
- Site-directed mutagenesis.
- Nick repair in duplex DNA, RNA or DNA/RNA hybrids.
- Self-circularization of linear DNA.
- Ligase-mediated RNA detection.

**One CEU is quantified as the quantity of enzyme required to achieve a 50% ligation of HindIII fragments from lambda DNA within a 30 min at a temperature of 16°C.*

Troubleshooting:

For troubleshooting, please email us at techteam@nextgenlife.com; info@nextgenlife.com.