



Description

DNA Ligation Kit

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The DNA Ligation Kit provides the necessary components for convenient, reproducible ligation of DNA fragments. The kit is optimized and tested for cloning cDNA and PCR products into plasmid and phage lambda vectors, and for linker ligations. Separate solutions of DTT and ATP are provided to ensure optimal performance and stability. Enough components are provided for 50 blunt-end or T-clonings, or up to 500 2-4 base cohesive end ligations.

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Components

- 100U T4 DNA ligase
- 500µl Ligase Dilution Buffer (20mM Tris-HCl pH 7.6, 60mM KCl, 5mM DTT, 0.5mg/ml BSA, 50% glycerol)
- 500µl 10X Ligation Buffer (10X = 200mM Tris-HCl pH 7.6, 100mM MgCl₂, 250µg/ml acetylated BSA)
- 250µl 100mM DTT
- 250µl 10mM ATP
- 1ml Nuclease-free water

Insert and Vector Preparation

Recommended conditions for use of the DNA Ligation Kit in three different applications are presented below. In general, optimal ligation is achieved starting with insert and vector DNA in TE buffer or water, although other conditions often give equivalent results. The components in most restriction enzyme buffers will not interfere with ligation, especially if the volume added to the reaction is minimized (given that appropriate steps have been taken to eliminate enzyme activity when required). Additional components that do not appear to have a deleterious effect are tRNA (up to 200µg/ml), glycogen (up to 200µg/ml), and NaCl (up to 300mM). The reaction will tolerate small amounts of dNTP, but [dATP] should be kept below 50µM.

T-Vector Ligations

DNA having single 3' dA overhangs as produced by PCR* amplification with exonuclease-deficient DNA polymerases are suitable for direct ligation with T-Vectors, which contain compatible single 3' dT overhangs.

Direct Cloning of PCR Products Without Purification

The need for PCR product purification will be determined by the quality of the amplified material. If the PCR was very clean (*i.e.* the gel shows a clear, distinct band of the desired size with little extraneous material), a small sample (*e.g.* 1µl) of the reaction can be added directly to the T-Vector ligation. To avoid the possible generation of false positives by residual polymerase activity, the enzyme should be inactivated before removing a sample for ligation. This can be accomplished by extracting the reaction with 1 volume of CIAA (chloroform:isoamyl alcohol, 24:1). Add the CIAA, vortex for 1 minute, centrifuge at 12,000 × g for 1 minute, and add up to 2µl of the aqueous phase to the ligation. Precipitation is not necessary unless the PCR product is at a low concentration.

Purification of Desired Product (Optional)

(Other methods of partial purification such as spin columns may be substituted.)

1. After the amplification reaction add 100µl of chloroform to remove the top oil phase, and then add 5µl of 10X gel loading dye and load onto a 1.5% agarose gel containing 0.5µg/ml ethidium bromide. Run at 5-10 volts per cm. Load Novagen's PCR Markers (Cat. No. 69278-3) or Perfect DNA™ Markers (Cat. No. 69002-3) in an adjacent lane.
2. Visualize the DNA band with a long wave UV light source and cut the band from the gel using a clean razor blade.



3. Recover the DNA from the gel slice using your favorite method. Resuspend the final product in a total volume of 10 μ l (usually about 20ng/ μ l).

Note: when using PCR to subclone into T-Vector from ampicillin resistant plasmid templates, it is necessary to gel purify the fragment of interest to remove the original plasmid, which will transform very efficiently. As little as 10pg of contaminating supercoiled plasmid can typically result in several hundred white colonies when using NovaBlue competent cells.

Ligation to T-Vector

Follow the protocol supplied with your T-vector kit.

*PCR is covered by U.S. Patent Numbers 4,683,195 and 4,683,202 owned by Hoffmann-La Roche.

Ligations Involving 2–4 Base Overhangs

Ligations of DNA having 2–4 base “sticky” ends require only about one-tenth the amount of ligase needed for blunt ends or T-Vector ligations. The DNA Ligation Kit includes a vial of Ligase Dilution Buffer for the convenient preparation of diluted enzyme to be used for this application. Enzyme diluted 10-fold in this buffer is stable at -20°C for many months.

1. For a standard reaction, 50ng (0.03pmol) of a 3kbp plasmid vector or 0.5 μ g (0.018pmol) of a lambda vector (*e.g.* λ SCREEN™ Vector Arms) is ligated with ~0.2pmol (50ng of a 500bp fragment) insert in a volume of 10 μ l. Assemble the following components in a 1.5ml tube.
1 μ l 10X Ligase Buffer
0.5 μ l 100mM DTT
1 μ l 10mM ATP
1 μ l 50ng/ μ l plasmid vector or 0.5 μ g/ μ l lambda vector arms
1 μ l 1/10X T4 DNA Ligase diluted in Ligase Dilution Buffer (0.2-0.3 Weiss units)
X μ l Target DNA (~0.2pmol)
Y μ l water
10 μ l
2. Add the ligase last and gently mix by stirring with a pipet tip. Incubate at 16°C 2 hr to overnight. Ligations can be stored at 4°C after heating at 70°C for 10 min to inactivate the enzyme.
3. Use ligation mixture directly for transformation or packaging.

Ligation of Blunt Ends

Two modifications to “sticky” end conditions are necessary for efficient ligation of blunt-ended linkers (and for other blunt-end ligations). First, the reaction is much less efficient, thus a large excess of linkers (or blunt-ended insert) and a high concentration of ligase is needed. Second, blunt ended ligation is inhibited at [ATP] above 0.5mM.

A notable exception is the blunt ended ligation involved in recircularizing plasmid DNA. This reaction is extremely efficient and can be accomplished using the conditions described above for 2-4 base overhangs, except that the [ATP] should be decreased to 0.1mM. Furthermore, this reaction appears to be enhanced by adding 5% PEG, although it should not be necessary because it is already very efficient.



Linker Ligations

1. For a standard reaction, 500ng target DNA (*e.g.* cDNA, average size 1.5kbp = ~0.625pmol) is ligated with 50pmol linkers in a volume of 10 μ l. Assemble the following components in a 1.5ml tube.
 - 1 μ l 10X Ligase Buffer
 - 0.5 μ l 100mM DTT
 - 1 μ l 1mM ATP (dilute 10mM stock to 1mM with water)
 - 1 μ l Target DNA (~0.625pmol)
 - 1 μ l T4 DNA Ligase (2-3 Weiss units)
 - X μ l Phosphorylated linkers (50pmol)
 - Y μ l water
 - 10 μ l
2. Add the ligase last and gently mix by stirring with a pipet tip. Incubate at 16°C 6 hr to overnight. Ligations can be stored at 4°C after heating at 70°C for 10 min to inactivate the enzyme.
3. Use ligation mixture directly for transformation or packaging.

Blunt-Ended Insert Ligation with Plasmid Vectors

The same conditions as above can be used, except that the amount of vector should be adjusted to about 0.03pmol and the amount of insert should be adjusted so that there is a 50–100 fold molar excess relative to the vector.