

Product:	DNA Ligation Kit
Description:	The principal ingredient of the DNA Ligation Kit is a mutated form of T4 DNA Ligase, the scope of which is to promote the ligation of DNA rapidly and effectively, both in cohesive and in blunt-ended varieties. This enzyme has the catalytic property of causing 2 strands of DNA to join up, between the 5'-phosphate and the 3'-OH groups of adjacent nucleotides, in either a blunt or cohesive-ended configuration.
Components	Cat No: CB-4427-3 50 Reactions 50µl Ligase 250µl 4x Buffer Cat No: CB-4428-3 100 Reactions 100µl Ligase 0.5 ml 4x Buffer
Performance	In 5 minutes, it is possible with 1µl of the DNA Ligase in a 20µl final reaction mix, to ligate either 99% of sticky (cohesive)-ended fragments of λ Hind III (100ng), or 80% of blunt-ended fragments of λ EcoR V (same quantity). However, for best results with blunt-ended ligations, a 15-minute incubation is suggested.
Content:	The DNA Ligase is accompanied by an appropriate quantity of 4 x DNA Ligation Buffer, which has been created expressly for use in this kit.
Storage & Dilution Buffer	10 mM Tris-HCl, pH 7.4, 100 mM NaCl, 1 mM DTT, 0.1 mM EDTA and 50% glycerol.
Method:	<ul style="list-style-type: none">- Before use, the buffer has to be vortexed.- Prior to ligation, the sample of DNA will need to be purified by means of spin column purification, or by phenol/chloroform extraction and ethanol precipitation.- The ingredients are mixed together in the following sequence at room temperature:<ul style="list-style-type: none">• 14µl of the DNA being ligated (if necessary diluted with water, e.g. x µl of the vector and y µl of the insert DNA). Each reaction should contain a maximum of 100ng of Total DNA.• 1 µl of QS DNA Ligase• 5 µl of the BufferThe entire mixture to be mixed by pipetting.- In order to avoid self-ligation, in the case of blunt-ended reactions, dephosphorylating the vector should be considered.- It is possible to determine the ideal ratio of vector-insert or phage-insert empirically, but in general, in respect of vector:insert ligations, the molar ratio would be 1:3, and in respect of vector arm:insert phage ligations, the molar ratio would be 8:1.- The ligated DNA can generally be used without the need to inactivate the DNA ligase. However, the DNA ligase can be inactivated if desired by raising the temperature of the mixture to 65°C for 10 minutes.
Caution:	Repeated melting and refreezing of the product in stock is detrimental, and should be kept to a minimum.
Q/C Assay Conditions:	Each batch of DNA Ligation Kit is tested for ligation to a single band, of the products of Lambda DNA cut both by <i>HindIII</i> and by <i>EcoRV</i> . The ligated DNA is then re-cut to ensure that the restriction pattern has not been altered.
Storage temperature:	Storage: At -20°C: Can be used for 6 months At +4°C: Can be used for 1 month
Batch details:	Batch No: See vial Units per vial: See vial
References:	1. Enrel M.J. and Richardson CC (1982) <i>The Enzyme</i> 15,3

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This certificate is a declaration of analysis at the time of manufacture