

QUALITY CONTROL FOR MODIFYING ENZYMES

Unit definitions

Unit definition varies from enzyme to enzyme and is given in the product description of this catalogue.

Performance Test

Each enzyme is tested for its performance using tests that are mentioned in product description.

Assay for non-specific endonuclease

All modifying enzymes are checked for the absence of non-specific endonuclease activity by incubating excess of the enzyme with supercoiled plasmid DNA for several hours and determining the level of nicking in supercoiled form. The highest number of units producing unaltered pattern is reported on the data sheet supplied with the enzyme.

Assay for exonuclease activity

The absence of exonuclease activity is checked for all the enzymes by incubating the enzyme with lambda/*Hind* III digest for several hours and running on an agarose gel. The sharp pattern indicates the absence of exonuclease. The highest number of units producing unaltered pattern is reported on the data sheet supplied with the enzyme.

Assay for RNase

The absence of contaminating RNases is tested by incubating total RNA from HeLa cells with excess of enzyme for 4 hours at 37°C and checking for any degradation on gel.

Purification

All modifying enzymes are purified to near homogeneity using the procedures developed at Bangalore Genei.

Stability

All batches of modifying enzymes are routinely tested for stability by checking unit activity. Most of the enzymes are stable for more than 12 months when stored at -20°C. Exposure to temperatures greater than -20°C should be minimised whenever possible.

T4 DNA Ligase

Description: T4 DNA ligase catalyses the linkage of adjacent 5'-phosphate and 3'-hydroxy ends of double stranded DNA by the formation of phosphodiester bond.

Unit Definition: One cohesive end ligase unit is defined as the amount of enzyme required to give 50% ligation of Lambda/*Hind* III digest in 30 minutes at 16° C in 20 µl of the reaction mixture and a 5' DNA termini concentration of 0.12 µM.

Relation to other Ligase Units: One cohesive end Ligase unit approximately equals 0.015 ATP-PP exchange unit (Weiss Unit).

Storage Buffer: 10 mM Tris-HCl (pH 7.5), 0.1 mM EDTA, 50 mM KCl, 1 mM DTT, 50% Glycerol and 200 µg/ml Nuclease free BSA.

Store at - 20°C

Note:

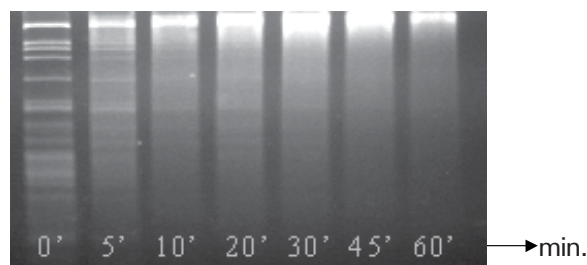
T4 DNA Ligase is supplied with 1 vial each of Cohesive & Blunt end Buffer.

For Buffer Composition refer Buffer Set for Ligation given on page no C62.

Application: This enzyme seals nicks in double stranded DNA and covalently joins DNA fragments with cohesive ends or blunt ends. This enzyme does not act on single stranded DNA.

Performance Test:

1. T4 DNA Ligase is routinely used in-house for quality control checks of all our cohesive and blunt end generating restriction enzymes. It is also used in the **Blue-White standard assay**.
2. Enzyme is tested for its performance in **cloning** experiments.



Blunt End Ligation

Ligation of λ/*Hae* III blunt ended fragments using 1 µl of T4 DNA ligase at 22°C at different time points from 0-60 minutes.

Ordering Information:

Product	Size	Cat #
T4 DNA Ligase	8000 U	105905
	40000 U	105904
	100000 U	105903
(High Conc.)	100000 U	105902