Link-FAST™ 5 Minutes DNA Ligation Kit

(Catalog #K902-50; Store kit at –80° C)

I. Introduction:

The Link-FAST™ 5 minutes DNA Ligation Kit provides a fast and convenient means for ligation of sticky-end or blunt-end DNA fragments in just 5 minutes at room temperature. All necessary reagents required for ligation are provided. There is no need to prepare buffers or to add ATP or Mg^{2+}. The kit can be used for cloning in plasmid vectors or phage vectors, linker ligation, recirculation of linear DNA, etc. Ligated DNA can be easily analyzed by agarose gel electrophoresis.

II. Kit Contents:

Component	K902-50	Cap Code	Part Number
DNA Dilution Buffer	0.5 ml	Clear	K902-50-1
2X T4 DNA Ligation Buffer	0.5 ml	Yellow	K902-50-2
T4 DNA Ligase (5 µg/ µl)	50 µl	Green	K902-50-3

III. Ligation Procedure:

A. General Consideration

- For insertion of DNA into plasmid vectors the vector DNA should be dephosphorylated (except for recirculation) with alkaline phosphatase.
- Dissolve the vector and insert DNA to be ligated in DNA Dilution Buffer to a total volume of 10 µl.
  Note: If the total volume of DNA is greater than 10 µl, then the volume of all the reagents in the reaction should be increased accordingly and the ligation reaction should be incubated for 30 minutes.
- T4 DNA ligase can be completely inactivated by a 10 min incubation at 65° C.
  Note: This step should only be done if the ligation reaction mixture is used in experiments other than transformation assays. Heat inactivation of the ligation reaction mixture before transformation leads to a drastic decrease of transformed colonies.
- It is absolutely necessary to thoroughly mix the contents of the kit reagents prior to use.

B. Ligation Procedure:

- Dissolve vector DNA and insert DNA in DNA Dilution Buffer. The molar ratio of vector DNA to insert DNA in a total volume of 10 µl should be 1:3 (e.g., as described in the standard assay with approximately equal length of vector DNA and insert DNA, 50 ng linearized dephosphorylated vector DNA and 150 ng insert DNA).
- When the vector DNA and insert DNA are not similar in lengths, other molar ratios 1:1, 1:2 are possible. A molar ratio of 1:5 can be used for sticky-end ligations. For blunt-ended DNA, however, a decrease of transformed colonies was observed when a molar ration of 1:5 was used.

  Note: To avoid an inhibition by a surplus of DNA, a maximum of one-tenth of the volume of the ligation reaction mixture should be used for the transformation assay. The maximum amount of DNA to be ligated in 5 min should not exceed 200 ng.

IV. Ligation Reaction:

- Add vector and insert DNA into an eppendorf tube, add DNA Dilution Buffer to 10 µl.
- Add 10 µl of 2X T4 DNA ligation buffer.
- Mix thoroughly and spin briefly.
- Add 1 µl of T4 DNA ligase.
- Mix thoroughly.
- Incubate for 5 minutes at room temperature.

Note: The ligation reaction mixture can be used directly for the transformation of competent cells, or can be stored without heat inactivation at -20° C. Heat inactivation of the T4 DNA ligase drastically decreases the transformation efficiency.

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