SpeedyLink Ligation Kit

Cat. No.	size
E1055-01	30 reactions
E1055-02	150 reactions

SpeedyLink Ligation Kit enables sticky-end or blunt-end ligation in only 5 minutes at room temperature (25°C). Fast ligation is based on the combination of T4 DNA Ligase and specially formulated buffer. It increases efficiency of ligation to that obtained with T4 DNA Ligase in a standard 1 hour ligation. After 5 min incubation and 15 min inactivation DNA is ready to transform into competent cells.

Applications:

- Cloning into vectors
- Library construction
- TA cloning
- Linker ligation
- Recircularization of linear DNA

Kit components:

SpeedyLink Ligation Kit	E1055-01	E1055-02
T4 DNA Ligase	30 µl	150 µl
2 x Ligation Buffer	300 µl	1.5 ml

Source: *E. coli* strain expressing a recombinant g30 gene of bacteriophage T4.

Storage Conditions: Store at -20°C.

2 x Ligation Buffer:

60 mM Tris-HCl (pH 7.5 at 25°C), 20 mM $MgCl_2$, 20 mM dithiothreitol, 14% PEG 6000, 2 mM ATP.

Avoid multiple cycles of freezing/thawing of the reaction buffer. Thawing should be performed at temperatures not exceeding 10°C. Recommended procedure is to divide the provided reaction buffer into smaller portions. DTT in the Reaction Buffer may precipitate upon freezing, if this occurs vortex the buffer until the precipitate dissolves.

Enzyme Storage Buffer:

10 mM Tris-HCl (pH 7.5 at 22°C), 1 mM DTT, 50 mM KCl and 50% (v/v) glycerol.

SpeedyLink Ligation Protocol:

Mix the following	
Linearized vector DNA	10-100 ng
Insert DNA	variable
2 x Ligation Buffer	10 µl
T4 DNA Ligase	1 µl
Water, nuclease free	Up to 20 µl

1. Mix 50 ng of the vector with a 3x molar excess of the insert. Adjust the volume to 9 µl.
2. Add 10 µl of 2 x Ligation Buffer.
3. Add 1 µl of T4 DNA Ligase and mix by pipetting.
4. Spin briefly and incubate at room temperature (25°C) for 5 minutes.
5. Dilute reaction (20 µl) with 40 µl of water and inactivate 15 min 65°C. Use 1-2 µl for transformation.

Notes:

- Dilution is necessary to reduce PEG concentration.
- Thermal inactivation is essential for transformation efficiency.

Quality Control:

All preparations are tested for contaminating endonuclease, exonuclease and non-specific single- and double-stranded DNase activities. The preparation is approximately 95% pure as judged by SDS polyacrylamide gel electrophoresis.

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Calculation of Required Vector and Insert DNA Solution Volumes.

Since ligation efficiency benefits from high initial DNA concentrations, the reaction is set up ideally without any diluting H_2O. For a quick calculation of the optimally required vector and insert DNA solution volumes, a formula was devised by Cranenburgh (2004).

1)

$$V_v = \frac{T}{\left(\frac{V_C \cdot I_l \cdot R}{I_C \cdot V_l}\right) + 1}$$

Example:

Component:	Example Value:
Insert length (I_l)	1.8 kb
Insert concentration (I_c)	20 ng /µl
Insert/ vector ratio (R)	3
Vector length (V_l)	3.2 kb
Vector concentration (V_c)	50 ng /µl
Total DNA volume (T)	8 µl
Vector volume (V_v)	to determine

$$V_v = \frac{8\ \mu l}{\left(\frac{50\ \frac{ng}{\mu l} \cdot 1.8\ kb \cdot 3}{20\ \frac{ng}{\mu l} \cdot 3.2\ kb}\right) + 1} \sim 1.5\ \mu l$$

2)

$$I_v = T - V_v$$

Example:

Component:	Example Value:
Vector volume (V_v)	1.5 µl
Total DNA volume (T)	8 µl
Insert volume (I_v)	to determine

$$I_v = 8\ \mu l - 1.5\ \mu l = 6.5\ \mu l$$

Notes:

1. **Prerequisites for efficient ligation and transformation:**
   - Well-purified DNA solutions of linearized vector and of insert DNA is an important factor for successful ligation.
   - To avoid damage to DNA fragments by UV light, we recommend visualizing and purifying these products by agarose gel electrophoresis using crystal violet (Rand, 1996). Crystal violet is non-mutagenic and easy to use. In addition, DNA can be visualized under normal light as a thin violet band while the gel is running and excised as soon as bands are sufficiently resolved
   - High DNA concentrations of linearized vector and insert DNA solutions (recommended 5-50 ng/µl) favour intermolecular over intramolecular (self-) ligation. Extremely high DNA concentrations lead to undesired formation of very long linear DNA fragments.
   - Electroporation can increase transformation efficiency by several logs. Before electroporation we recommend reaction dilution and thermal inactivation or spin column purification.
   - Quality of competent cells directly correlates to perceived ligation efficiency.
2. **Reaction speed:** Velocity of the ligation reaction depends solely on the concentration of free, compatible DNA ends, regardless whether they are located on the same DNA strand (intramolecular ligation) or on different DNA strands (intermolecular ligation). Two factors favouring intermolecular ligation over self-ligation are high DNA concentrations and long DNA fragments. Contrary, low DNA concentrations and small DNA fragments lead to a preference for self-ligation. Under the latter conditions it is more likely that two ends from one single molecule, rather than from different DNA strands, will get into close spatial contact.
3. **Dephosphorylated DNA:** Strategy to prevent self-ligation, is to remove 5'-phosphates from plasmid DNA (but not from insert DNA) prior to ligation. Bacterial and Calf Intestine Phosphatase (Cat. No. E1027 and E1025) catalyse the removal of 5'-phosphate groups from DNA and RNA. Dephosphorylation of plasmid DNA fragments efficiently prevents self-ligation, at the expense, that only two new phosphodiester bonds are formed during ligation (not four, as for phosphorylated DNA strands). Ligated molecules thus carry two nicks, which are repaired by the bacterial host following transformation.
4. **Optional Control Reactions:**
   - Cut vector, no insert - for estimating self-ligation background (blunt end: plus checking for ligatability).
   - Cut vector, no ligase - for estimating undigested vector background.
   - Uncut vector – for estimating efficiency of transformation.

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