EURx® MOLECULAR BIOLOGY PRODUCTS

# LigON Kit

| Cat. No. | size |
|---|---|
| E1050-01 | 10 reactions |
| E1050-02 | 5 x 10 reactions |

## Storage Conditions:

Store at -20°C.

Avoid multiple freeze and thaw cycles by preparing aliquots of the LigON Master Mix.

## Components:

1. LigON Master Mix (2x)
2. Positive control
3. Nuclease-free water

## Required Reagents Not Included:

- DNA polymerase for generating PCR products (Hybrid DNA Polymerase, E2950).
- DNA Purification Kit for DNA clean up (E3520, E3540).
- Competent Cells
- SOC Outgrowth Medium
- LB Luria Plates
- DpnI (E2135)

## Notes:

1. Positive control consists of a mixture of vector and insert. Select assembled product on LB agar plates with 100 µg/ml ampicillin, 0.1 mM IPTG. Positive clones should glow green under UV light.
2. We recommend using electroporation due to higher transformation efficiency.
3. Assembly performance depends on:
   - the length of fragments being assembled,
   - number of fragments being assembled,
   - quality of DNA,
   - quality of competent cells,
   - method of transformation.

**LigON Kit is designated for assembling multiple DNA fragments in one single isothermal reaction.** This simple one tube, one step, sequence-independent isothermal reaction allows to create large recombinant DNA constructs, with multiple inserts.

LigON Kit utilizes a special mix of enzymes optimized to assemble the DNA quickly and efficiently. This method eliminates restriction enzymes, introducing any extra codons or scars. This results in higher numbers of error free clones ready for multiple downstream applications.

## Protocol:

1. Design primers for DNA fragments and vector. Make sure all fragments to be joined have overlapping sequences (20-100 bp) at the ends. Complementary sequences determine the order in which DNA fragments are assembled. Potential secondary structures in overlapping regions may affect a proper hybridization of homologous sequences.
2. Amplify DNA fragments by PCR using high fidelity polymerase.
3. Clean up all DNA substrates and vector (use DpnI restriction enzyme to remove the plasmid template DNA).
4. Determine DNA concentration of all DNA fragments.
5. Mix all DNA compounds and LigON Master Mix:

| Reaction components | 2-3 Fragment Assembly | 4-6 Fragment Assembly | Positive Control |
|---|---|---|---|
| Total amount of fragments | 0.02-0.5 pmol x µl | 0.2-1 pmol x µl | 10 µl |
| LigON Master Mix (2x) | 10 µl | 10 µl | 10 µl |
| Nuclease-free Water | 10-x µl | 10-x µl | 0 |
| Total Volume | 20 µl | 20 µl | 20 µl |

6. Incubate reaction at 50°C for 30 min up to 1 hour depending on number of fragments being assembled.
7. Stop reaction on ice or store at -20°C for further applications.
8. Dilute reaction with deionized water 1:3 (20 µl + 40 µl H2O).
9. Use 1 µl of diluted reaction to transform competent cells.
10. Spread 100 µl of the cells onto the plates with appropriate antibiotics. Use ampicillin/IPTG plates for positive control sample.
11. Incubate plates at 37°C overnight and check for positive clones. Positive control should yield about 100 colonies when electroporation is used as a transformation method.

## Quality Control:

All preparations are assayed for contaminating unspecific exonuclease and double-stranded DNase activities.



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