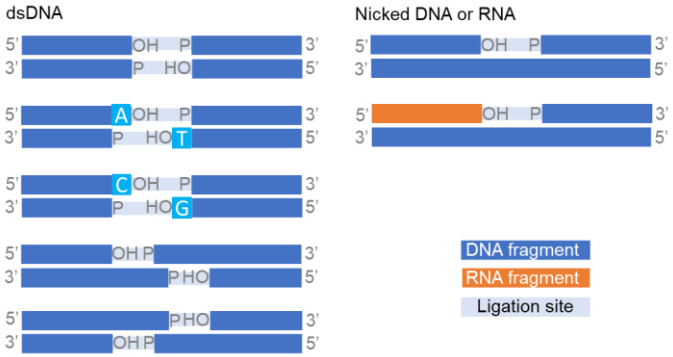
Rapid Ligation Master Mix

Catalog No. 40102S: 30 reactions
Catalog No. 40102L: 150 reactions

Description

The **Rapid Ligation Master Mix** is optimized for DNA fragment ligation of sticky end or blunt end in only 5 minutes. The ligation reagent is a 2X master mix containing T4 DNA Ligase and proprietary ligation enhancers. The components of the master mix improve the ligation efficiency significantly when using different types of double-stranded DNA fragments.

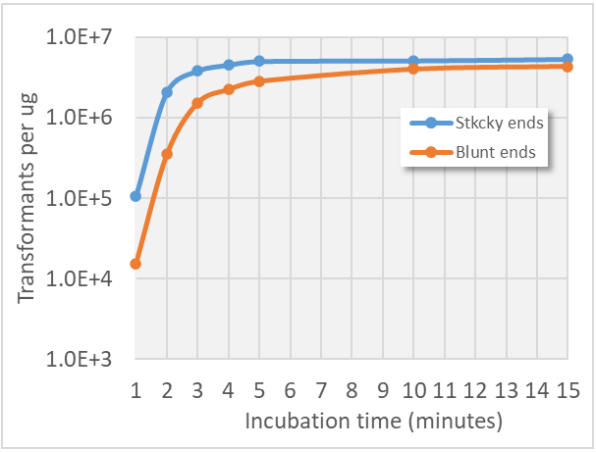
The **Rapid Ligation Master Mix** can be used for the ligation of the following fragments:



The **Rapid Ligation Master Mix** makes it easy to set up ligation reactions by simply mixing the DNA fragments with the 2X Master Mix. The ligation of all types of DNA ends is fast at room temperature with high efficiency. The subsequent transformation protocol is also simplified to save both time and efforts.

Features

- Fast ligation: only 5 minutes for sticky ends or blunt ends
- Room temperature (20-25°C) ligation
- Easy ligation reaction setup with the 2X Master Mix format
- Small volume of reaction made cloning possible with limited DNA input
- Simplified transformation protocol



Ligations with sticky ends or blunt ends using the **Rapid Ligation Master Mix** for different times at 20°C. The ligation plasmids were transformed into chemical competent cells DH-5α and grown on LB plates with carbenicillin at 37°C overnight.

Component

Catalog No.	40102S	40102L
Rapid Ligation Master Mix (2X)	150 ul	750 ul

Storage Condition

- Store at -20°C, stable up to 12 months.

Reagent & Equipment Needed (not provided in this kit)

- Agar plates containing antibiotics
- 42°C water bath
- 37°C shaking and non-shaking incubator
- General microbiological supplies (e.g., plates, spreaders)
- Competent cells for transformation

Protocol

Ligation Protocol

1) Thaw the Master Mix on icy water if the mix is frozen. Tapping the tube to mix before use.
2) Mix 20-100 ng of vector with a 3-fold molar excess of inserts. Adjust the volume to 5 µl with water.
3) Add 5 µl of the Master Mix and mix thoroughly by pipetting up and down around 10 times or by tube tapping. Store the Master Mix at -20°C immediately after use.
4) Incubate at room temperature (20°C-25°C) for 5 min. **Note**: extended incubation time up to 15 min may increase the efficiency slightly.
5) Continue with transformation protocol or store samples at -20°C. **Note**: Do not heat inactivate. Heat inactivation reduces transformation efficiency.

Transformation Protocol (Recommended)

Chemically competent strains of E. coli can be used. Electrocompetent cells are not compatible. If the transformation efficiency is low, dilution of the ligation reactions 4-fold may be needed prior to transformation. The volume of ligation reaction used should not exceed 10% of the competent cells.

1) Thaw competent cells on icy water.
2) Add 50 µl of competent cells into a 1.5 ml tube.
3) Add 2 µl of the ligation reaction to the tube and mix by tapping the tube gently. Do not vortex.
4) Incubate the tube on ice for 30 minutes. Do not mix.
5) Heat the tube at 42°C for 40 seconds, then place it on icy water for 2 minutes.
6) **Optional**: Add 950 µl recovery media (e.g. SOC) in the tube and incubate for one hour at 37°C with shaking at 200–250 rpm. This step can be skipped although the colony numbers maybe slightly lower.
7) Spread 50 µl of the bacteria onto plates with appropriate antibiotic selection. Incubate overnight at 37°C.

Note: Many factors can affect the transformation efficiency, including media type, integrity and purity of DNA fragments, vector type, vector size, competence of the E. coli cells used, insert DNA fragment size, incubation time and incubation temperatures, and copy numbers etc.

Troubleshooting

Problem: Few or no colonies

Possible cause	Solution
Impurity of DNA.	Purify DNA using column or beads to remove contamination.
DNA degraded or insufficient DNA.	Check DNA by gel electrophoresis. Determine DNA concentration and add the correct amount. Use the supplied positive control to test the system.
Incorrect amounts of antibiotics or wrong antibiotics was used in agar plates.	Check the correct amount of antibiotics was used in agar plates. Do not spread antibiotics onto the surface of agar plates.
The transformation efficiency of competent cells is too low.	Use competent cells with high transformation efficiency. Check the transformation efficiency of competent cells.

Problem: High background of colonies that do not contain inserts.

Possible cause	Solution
Non-specific DNA or plasmid contamination in ligation.	Gel purify DNA before ligation.
Enzyme contamination in ligation.	Purify DNA using column or beads.
Incorrect amounts of antibiotics or wrong antibiotics were used in agar plates.	Check the correct amount of antibiotics was used for agar plates. Do not spread antibiotics onto the surface of agar plates.

Quality Control

Reagent passed stringent functional quality test.

Product Use Limitation

This product is developed and sold for research purposes and *in vitro* use only. Please refer to biodynami.com for Material Safety Data Sheet of the product.

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