Super Ligation Master Mix

Cat.:BR1C302

V01

Product Description

Super Ligation Master Mix is a ready-to-use 2× premixed solution containing T4 DNA Ligase and buffer. It catalyzes phosphodiester bond formation between adjacent 5'-phosphate and 3'-hydroxyl termini on double-stranded DNA or RNA. It is suitable for ligation of sticky ends, blunt ends, and single-base overhang DNA, as well as linker or adapter ligation. Rapid ligation can be completed in 30 min at 16°C, and the ligation products can be directly transformed into chemically competent cells.

Components

Components	BR1C302-01 20 T	BR1C302-01 50 T	BR1C302-01 100 T
Super Ligation Master Mix	0.1 mL	0.25 mL	0.5 mL

Storage

Store at -20±5°C.

Notes

1.For Research Use Only. Not for use in diagnostic procedures.

2.Please thaw and mix thoroughly before use. Avoid repeated freezing and thawing.

3.In general, the recommended vector amount (0.03 pmol) = [0.02 × vector length in bp] ng, and the recommended insert amount (0.09 pmol) = [0.06 × insert length in bp] ng.

4.To ensure accurate pipetting, the linearized vector and insert may be diluted before reaction setup so that each component is added at no less than 1 μL.

5.After the reaction, place the mixture on ice and transform immediately. If immediate transformation is not possible, store at -20°C.

Protocol

Ligation Reaction

Reaction setup: It is recommended to prepare the ligation reaction on ice.

Component	Volume per Reaction
Super Ligation Master Mix	5 μL
Linearized Vector	X μL
Insert Fragment	Y μL
ddH2O	To 10 μL

X and Y indicate the calculated optimal amounts of linearized vector and insert. To ensure accurate pipetting, dilute concentrated samples as needed so that each component is added at no less than 1 μL.

Calculation of linearized vector and insert amounts: The recommended linearized vector amount is 0.03 pmol, and the insert amount is 0.03-0.3 pmol. Approximate amounts can be calculated using the formulas below. The molar ratio (vector:insert) ranges from 1:1 to 1:10, with 1:3 recommended as the optimal ratio. For larger fragments, the insert proportion may be increased as needed.

Component	Optimal Amount (ng)	Optimal Amount (pmol)	Molar Ratio (Vector:Insert)
Linearized Vector	[0.02 × vector length in bp] ng	0.03 pmol	1:3
Insert Fragment	[0.06 × insert length in bp] ng	0.09 pmol	

Ligation reaction conditions:

Standard Conditions	Optimization Range
16°C, 30 min	16°C, 30 min-3 h
	25°C, 5 min
	4°C, overnight

Transformation of Ligation Products

1. Thaw the competent cells on ice.
2. Add 5-10 μL of ligation product to 100 μL of competent cells. Gently flick to mix (do not vortex), and incubate on ice for 30 min (25 min for some competent cells). The ligation product volume should not exceed 1/10 of the competent cell volume.
3. Heat shock at 42°C for 30 sec (follow the instructions for the competent cells used), then immediately place on ice for 2-3 min.
4. Add 900 μL of antibiotic-free LB broth and shake at 37°C and 200-250 rpm for 1 h.
5. Centrifuge at 5,000 rpm for 5 min. In a clean bench, discard 900 μL of the supernatant, resuspend the cells in the remaining medium, plate onto LB agar plates containing the appropriate antibiotic, and incubate inverted at 37°C for 12-16 h.

Positive Clone Identification

Colony PCR: Pick a single colony into 10 μL sterile water as the amplification template. Use appropriate forward and reverse primers, with at least one vector-specific primer recommended.
Restriction analysis: Pick a single colony into LB broth containing the appropriate antibiotic, culture overnight, and isolate the plasmid. Digest using suitable double-digestion sites on the vector. Confirm positive clones by electrophoresis based on whether the fragment sizes match the expected sizes.
Sequencing analysis: Pick a single colony into LB broth containing the appropriate antibiotic. Either the culture or plasmid extracted from the culture may be submitted for sequencing. At least one vector-specific sequencing primer is recommended. Compare the sequencing results with the expected sequence to identify positive clones and evaluate any mutations introduced during cloning.

FAQ

No or Few Transformants on Plates

1. End incompatibility: Blunt and sticky ends may be mixed after digestion, or sticky ends may contain base mismatches. After double digestion, perform gel purification to ensure that the insert restriction sites match the vector ends. The double-digestion sites must fully match the vector multiple cloning site (MCS) and be in the correct orientation; otherwise, the fragment cannot be inserted correctly.
2. Imbalanced insert-to-vector ratio: Optimize the ligation ratio. The molar ratio (vector:insert) ranges from 1:1 to 1:10, with 1:3 recommended as the optimal ratio. For larger fragments, the proportion of insert may be increased appropriately.
3. Poor vector quality: Heat inactivate the restriction enzymes after vector digestion to avoid residual endonuclease activity that may damage the ligation products.
4. Low ligation efficiency: Try ligation at 16°C for 3 h while maintaining low temperature throughout the reaction. Extending the reaction time can improve ligation efficiency.
5. Low transformation efficiency of competent cells: The transformation efficiency of competent cells should be greater than 1 × 10^7 cfu/μg.

No Positive Clones or High False-Positive Rate

1. Incomplete double digestion of vector: Sequential digestion may be used. Optimize the restriction buffer system, for example with a compatible buffer such as CutSmart Buffer. Gel verification can also be performed by setting up single-digestion, double-digestion, and no-digestion controls and comparing band patterns after electrophoresis.
2. Inappropriate spacing between double-digestion sites: A safe distance should be reserved between the two restriction sites, with at least 10 bp between them.
3. Vector self-ligation: After double digestion, treat the linearized vector with alkaline phosphatase (such as CIP) to remove the 5'-phosphate groups and block self-ligation. Check whether the restriction sites used for double digestion are isocaudomers.
4. Inappropriate primers for fragment amplification: Ensure that the primers contain 5' protective bases plus terminal restriction sites. Avoid primer dimers or hairpin structures.