Servicebio® pSWE-Topo Zero Cloning Kit

Cat. #：G3022-25T

Product Information

Product Name	Cat. No.	Spec.
pSWE-Topo Zero Cloning Kit	G3022-25T	25 T

Product Description/Introduction

The pSWE-Topo Zero Cloning Kit produced by our company is a vector for screening positive recombinants by the expression of suicide gene. It is transformed from pUC19 vector. When the target gene fragment is not cloned into the vector, the suicide gene is successfully and correctly expressed, and the cells containing the vector cannot grow. Otherwise, the suicide gene will not be correctly expressed, and the cells containing the vector can grow normally. This vector is also known as the "Zero" background vector. The pSWE-Topo Zero Cloning Kit uses a new vector ligation method, which does not require additional ligase for the ligation reaction between the fragment and the vector. It only needs to add the target gene fragment to the vector to be reacted for 5 min at room temperature (22℃-37℃). The pSWE-Topo Zero Cloning Kit is compatible with TA cloning and blunt-end cloning.

Features: easy to use, compatible with TA cloning and blunt cloning; No ligase is required, the target gene fragment is added to the system, and cloning reaction can be performed for 5 minutes at room temperature; the positive cloning rate can reach 95%; suitable for short and long fragments; Selection marker: ampicillin; sequencing primers: M13 Forward Primer, M13 Reverse Primer.

Storage and Shipping Conditions

Ship with wet ice; store at -20°C, valid for 12 months.

Product Contents

Component Number	Component	G3022-25T
G3022-1	pSWE-Topo Zero Vector (50 ng/μL)	25 μL
G3022-2	Control Template (700 bp, 50 ng/μL)	10 μL
G3022-3	M13 Forward Primer (10 μM)	50 μL
G3022-4	M13 Reverse Primer (10 μM)	50 μL
Manual		One copy

Assay Protocol / Procedures

Prepare of Target Fragment

1. PCR amplification of target fragment

Note: (1) The primers cannot be phosphorylated; (2) High fidelity and Taq DNA Polymerase can be used for the PCR reaction; (3) It is recommended to use a gel recovery kit for purification of PCR products after electrophoresis.

Perform ligation reaction:

2. To an autoclaved, 1.5-ml microcentrifuge tube, add the following:

Component	Volume
pSWE-Topo Zero Vector (50 ng/ μL)	1 μL
The target gene fragment[a]	0.5-4 μL

Note: The recommended amount of insert: the molar ratio of vector to fragment = 1:10-1:3. It can be roughly calculated

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by adding 50 ng of 1 kb fragment. If a gene library is constructed, the reaction system can be appropriately expanded.

3. Mix gently and centrifuge briefly to bring the contents to the bottom of the tube.
4. Incubate at room temperature (20-37°C) for 5-10 minutes.
5. Place the tube on ice and Proceed immediately to "Perform transformation reaction".You can store the cloning reactions at –20°C until you are ready.

Perform transformation reaction

6. Add appropriate ligation reaction into Chemically Competent *E. coli* (such as DH5α, Top10, etc.)and mix gently. Do not mix by pipetting up and down.
7. Incubate for 30 minutes on ice.
8. Heat-shock the cells for 30 seconds in a 42°C water bath.
9. Immediately place the tubes on ice and incubate for 2 minutes.
10. Add 900 µL of room temperature SOC or LB medium. Cap the tube tightly and shake the tube horizontally at 225 rpm for 1 hour at 37°C.
11. Spread required volume of transformation reaction on a prewarmed LB plate containing corresponding antibiotics.
12. Incubate plates overnight at 37°C.

Analyze transformants

13. Pick an individual colony from the transformation plate, analyze transformants by colony PCR, restriction enzyme digestion or sequencing.

    (1) Colony PCR: pick an individual colony into 10 µL sterile water, mix by vortexing, then take 1 µL of the mixture as PCR template, M13 Forward Primer and M13 Reverse Primer as universal primers, and perform colony PCR positive identification clone. (recommended G3304, G3305; PCR reaction system and procedures refer to the corresponding product manual, positive clone PCR amplification fragment> 100 bp)

    (2) restriction enzyme digestion: pick an individual colony and inoculate it in an appropriate amount of resistant liquid medium, and cultivate overnight at 220rpm and 37°C. A small amount of plasmid was extracted, digested with appropriate restriction enzymes, and positive clones are identified by gel electrophoresis.

    (3) sequencing: use M13 Forward Primer and M13 Reverse Primer primers to sequence and analyze.

Note

1. It should be placed in an ice box with using this product, and it should be stored at -20°C immediately after use.
2. *Escherichia coli* strains DB3.1, ccdB Survival, Stable, JM109, XL1-Blue and XL10-Gold resistant to CcdB are not suitable for pSWE-Topo Zero Vector. Competent cells of *Escherichia coli* that are intolerant to Ccdb, such as DH5α, Top10, TG1, which are commonly used in other laboratories, are suitable.
3. For your safty and health,please wear safety glasses, gloves, or protective clothing.

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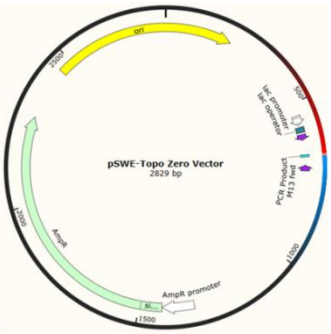
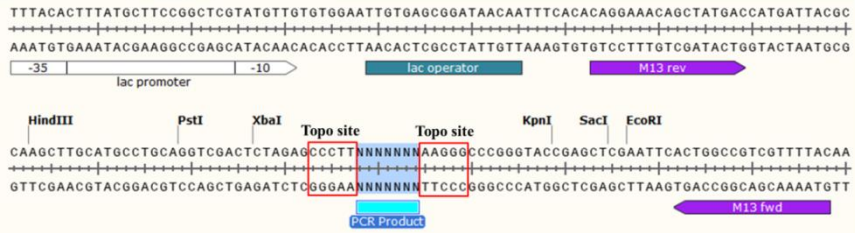


Lac promoter site：bases 541-571
M13 reverse priming site：bases 603-619
M13 Forward priming site：bases 702-718
Ampicillin resistance ORF：bases 1427-2287
pUC origin：bases 2458-217



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