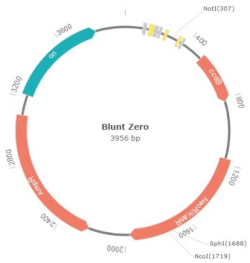
Blunt Zero Cloning Kit

Catalogue No.:abx098062



Plasmid map (Blunt Zero).

Blunt Zero Cloning Vector contains a suicide gene. Ligation of PCR fragment disrupts the expression of the gene. Cells that contain non-recombinant vector are killed upon plating. High cloning efficiency. Positive clones up to 100%. Suitable for larger fragment cloning. M13 forward primer and M13 reverse primer for sequencing. T3 promoter and T7 promoter for in vitro transcription.

Kit contents:

Component	**20 rxns**	**60 rxns**
Blunt Zero Cloning Vector (10 ng/µl)	20 µl	3 × 20 µl
Control Template (5 ng/µl)	5 µl	5 µl
Control Primers (10 µM)	5 µl	5 µl
M13 Forward Primer (10 µM)	50 µl	150 µl
M13 Reverse Primer (10 µM)	50 µl	150 µl
Phage Resistant Chemically Competent Cells (abx098070)	10 × 100 µl	30 × 100 µl

Target: Blunt Zero Cloning Kit

Storage: Store Phage Resistant Chemically Competent Cells at -70°C for up to 6 months and all other components at -20°C for up to 9 months.

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Directions for use:

Preparation

1. Add 0.5 µl - 4 µl of PCR products and 1 µl of Blunt Zero Cloning Vector (10 ng/µl) into a microcentrifuge tube.
2. Gently mix, incubate at room temperature for 5 minutes, and place the tube on ice.

The optimal molar ratio of the vector to insert is 1:7 (for example, 1 kb = 20 ng, 2 kb = 40 ng). The total reaction volume should be 3 µl - 5 µl. The incubation time should be optimised by the end user, and the following recommendations may be used as a guide:

- 0.1-1 kb: 5-10 mins
- 1-2 kb: 10-15 mins
- 2-3 kb: 15-20 mins
- ≥3 kb: 20-30 mins.

Note: If the insert is gel purified, use the maximum incubation time of 20-30 minutes.

Transformation

1. Add the ligated products to 50 µl of Phage Resistant Chemically Competent Cells and mix gently. Do not mix by pipetting up and down.
2. Place on ice for 20-30 minutes.
3. Heat shock the cells at 42°C for 30 seconds, then immediately place the tube on ice for 2 minutes.
4. Add 250 µl of room temperature SOC or LB Medium, then incubate in a shaking incubator (200 rpm) at 37°C for 1 hour.
5. Inoculate a pre-warmed culture plate with 200 µl of the transformants. Incubate at 37°C overnight.

Identification of positive clones

1. Transfer 5-10 white or light-blue colonies into 10 µl of Nuclease-free water and vortex.
2. Use 1 µl of the mixture as the template for 25 µl PCR using M13 forward and M13 reverse primers.

PCR Conditions

Number of Cycles	Temperature	Time per Cycle
1 cycle	94 °C	10 min
30 cycles	94 °C	30 seconds
30 cycles	55 °C	30 seconds
30 cycles	72 °C	Dependent on insert size and PCR Enzyme
1 cycle	72 °C	5-10 min

Sequencing analysis

Positive clones should be inoculated in Ampicillin or Kanamycin selective LB liquid media, then incubate in a shaking incubator (200 rpm) at 37°C for 6 hours. Isolate plasmid DNA for restriction enzyme digestion and DNA sequencing. Sequencing should be performed using M13 forward, M13 Reverse and T7 promoter.

Components required for PCR of control insert (700 bp)

Component	Volume	Final concentration
Control template (5 ng/µl)	1 µl	0.1 ng/µl
Control Primers (10 µM)	1 µl	0.2 µM
2X PCR SuperMix	25 µl	1X
Nuclease-free water	Variable	-
Total volume	50 µl	-

PCR Conditions

Number of Cycles	Temperature	Time per Cycle
1 cycle	94 °C	2-5 min
30 cycles	94 °C	30 seconds
30 cycles	50-60 °C	30 seconds
30 cycles	72 °C	1 min
1 cycle	72 °C	10 min

Notes

- The PCR Enzyme should be a *Pfu* DNA Polymerase.
- Primers must not be phosphorylated.
- 5-10 min post extension step is required. After amplification, agarose gel electrophoresis is recommended to validate the quality and quantity of the PCR products.

Note: This product is for research use only. This product is shipped with dry ice.

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