

## pEZ-M67 Vector

### ■ Description

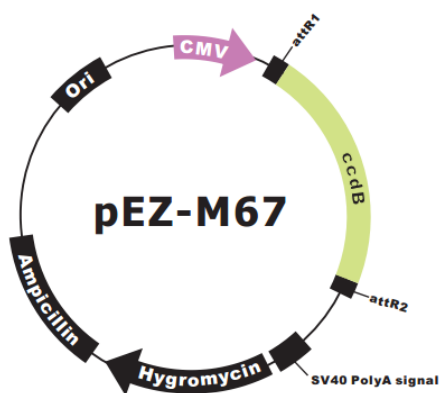
pEZ-M67 vector is adapted for use with the Gateway recombination technology. It has *attR* sites for recombination with an entry clone (containing a gene of interest flanked by *attL* sites). pEZ-M67 contains Ampicillin and Hygromycin selection markers. The CMV promoter, constitutive expression promoter downstream target genes. It expresses in mammalian cells, and can also be stable transfection for transient transfection.

### ■ Components Supplied

Component	Cat. No.	Unit size	Storage
pEZ-M67 Vector (150 ng/μl)		6 μg ×1	-20 °C

Storage time: 12 months

### ■ pEZ-M67 Vector Map



Vector	Promotor	Host cell	Transfection	Tag	Antibiotic
pEZ-M67	CMV	Mammalian	Hygromycin	N/A	Ampicillin

### ■ Sequencing primer

Primer	Sequence
Forward primer	5' - CAGCCTCCGGACTCTAGC-3'
Reverse primer	5' - TAATACGACTCACTATAGGG-3'

## ■ Procedures

### LR Recombination Reaction

Use the following procedure to perform an LR recombination reaction.

- 1 Add the following components to a 1.5 ml microcentrifuge tube at room temperature and mix:

Entry clone (100-300ng)	1-10 µl
Destination vector (150ng/µl)	2 µl
5 X Reaction Buffer	4 µl
TE buffer, pH 8.0	to 16 µl

- 2 Remove LR Recombinase Enzyme Mix from -80 °C and thaw on ice for about 2-3 minutes. Microcentrifuge briefly.
- 3 Add 4 µl of LR Recombinase Enzyme Mix to the reaction tube and mix well.
- 4 Briefly centrifuge to make sure all the reagents are at the bottom of the reaction tubes.
- 5 Incubate reactions at 25 °C for 60 minutes.
- 6 Return LR Recombinase Enzyme Mix to -80 °C storage immediately after use.

### Transformation

- 1 Thaw competent cells on ice. Place required number of polypropylene tubes on ice for transformation.
- 2 Transform 4 µl of each LR reaction into 50-100 µl of Competent Cells. Incubate on ice for 30 minutes.
- 3 Heat shock cells by placing the tubes into a 42 °C water bath for 30 seconds.
- 4 Immediately place the tubes on ice. Incubate for 2 to 3 minutes.
- 5 Add 400 µl of SOC medium and incubate at 37 °C for 1 hour with shaking at 200 rpm.
- 6 Plate 50 µl and 200 µl of each transformation onto antibiotic-containing plates.
- 7 Incubate plates for 12 to 16 hr at 37 °C.

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