

## pEZ-M02 Vector

### ■ Description

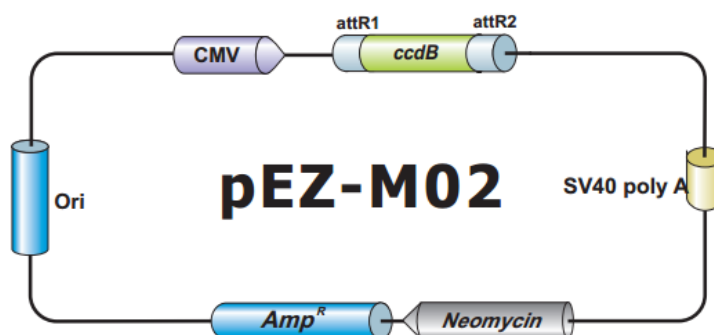
pEZ-M02 vector is adapted for use with the Gateway recombination technology. It has *attR* sites for recombination with an entry clone (containing a gene of interest flanked by *attL* sites). pEZ-M02 contains Ampicillin and Neomycin selection markers. The CMV promoter, constitutive expression promoter 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-M02 Vector (150 ng/μl) |          | 6 μg ×1   | -20 °C  |

Storage time: 12 months

### ■ pEZ-M02 Vector Map



| Vector  | Promotor | Host cell | Transfection | Tag | Antibiotic |
|---------|----------|-----------|--------------|-----|------------|
| pEZ-M02 | CMV      | Mammalian | Neomycin     | 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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