pEZ-Lv195 Vector

■ Description

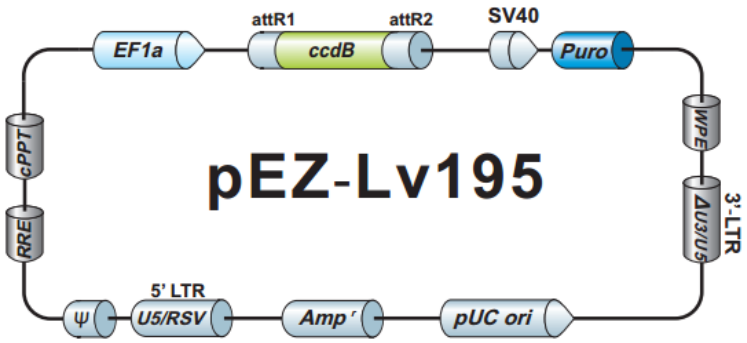
pEZ-Lv195 vector is adapted for use with the Gateway recombination technology. It has *att*R sites for recombination with an entry clone (containing a gene of interest flanked by *att*L sites). pEZ-Lv195 contains Ampicillin and Puromycin selection markers. The T7 promoter is a constitutive promoter, can express exogenous gene in bacteria. It expresses in a variety of stem cells and primary cells, and can also be stable transfection for transient transfection.

■ Components Supplied

Component	Cat. No.	Unit size	Storage
pEZ-Lv195 Vector（150 ng/μl）		6 μg ×1	-20 ℃

Storage time：12 months

■ pEZ-Lv195 Vector Map



Vector	Promotor	Host cell	Transfection	Tag	Antibiotic
pEZ-Lv195	EF1a (HIV RSV-LTR)	Stem/ primary cell	Puromycin	SV40-puromycin	Ampicillin

■ Sequencing primer

Primer	Sequence
Forward primer	5’-GTTTCGTTTTCTGTTCTGC-3’
Reverse primer	5’-CTGGAATAGCTCAGAGGC-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 ℃ 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 ℃ for 60 minutes.

6 Return LR Recombinase Enzyme Mix to -80 ℃ 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 ℃ 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 ℃ 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 ℃.

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