Authorized host vector system

Rhodococcus erythropolis L88 Competent Cells

Product No. RE-L88

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1. Description

Rhodococcus erythropolis L88 is a lysozyme-sensitive strain which was obtained by inducing point mutation through UV irradiation to *Rhodococcus erythropolis* JCM3201. Wild type is resistant to over 1 mg/ml lysozyme, whereas 12.5 μg/ml lysozyme cause bacteriolysis to *Rhodococcus erythropolis* L88. Therefore, it is easier to disrupt cells and extract protein than wild type. This strain can grow and express recombinant protein at 4 to 30 ° C. It is therefore expected to successfully express recombinant protein which is difficult in *E. coli*, such as lethal protein, protein inhibiting cell proliferation, and protein forming inclusion body.

2. Content

R. erythropolis L88 Competent Cells

Volume: 500 μl (100 μl ×5)

Storage: under -80 °C

In the case of improper thermal management, transformation efficiency may be decreased.

3. Transformation Efficiency

When 100 μl *R. erythropolis* L88 Competent Cells were transformed with 100 ng pCpiRC2 and then cultured on LB plate with chloramphenicol, its transformation efficiency was over 1×104 colonies/μg.

4. Operation Procedure

Transformation by Electroporation

1. Thaw *R. erythropolis* L88 Competent Cells on ice immediately before use.
2. Tap to mix uniformly.
3. Add 50-200 ng/μl plasmid DNA.
4. Incubate on ice for 30 minutes.
5. Electroporate at the condition of 1.6 kV, 25 μF, 400Ω in 1 mm cuvette.
   In the case of using other cuvette, modify amount of competent cells and condition of electroporation.
6. Add electroporated cells to 1 ml LB medium.
7. Incubate it with shaking at 28 °C for 60 minutes (recovery culture).
8. Culture on LB plate with an antibiotic (tetracycline: 5 μg/ml, chloramphenicol: 20 μg/ml)
9. Incubate at 28 °C. Colonies are formed in 2-3 days generally but it takes about 1 week in some cases.

Precautions

a. Since transformation efficiency of *R. erythropolis* L88 is lower than *E. coli*, these competent cells are not applicable to cloning and vector construction. It is recommended to construct vector in *E. coli* and then transform *R. erythropolis* L88 by using the plasmid.

b. Related products, pTip, pNit, pCpi Vector Series may be unstable according to the strain of *E. coli*. DH5α and XL1-blue are recommended.

c. It is not necessary to demethylate constructed vector.

5. Troubleshooting

If transformation efficiency is low, it may improve by one of the following changes:

a. Increase amount of competent cells.

b. Increase amount of vector.

c. Increase time of recovery culture to 2 hours.

6. References

1) Mitani et al. "Characterization of LtsA from *Rhodococcus erythropolis*, an enzyme with glutamine amidotransferase activity." Journal of bacteriology 187.8 (2005): 2582-2591.

7. Cautions

Reselling or modification of these products and producing any commercial products from these products are not permitted.

These products are for research use only.

These products, their main structures, their derivative and what is produced through them cannot be transferred to a third party.

It is necessary to submit "*Rhodococcus erythropolis* Protein Expression System License Agreement" when you order these products.

8. Related product

Rhodococcus Expression Vectors

pTip, pNit, pCpi Vector Series

pTip Vector Series(Product No.:RE-0001～RE-0008)

pNit Vector Series(Product No.:RE-0009～RE-0016)

pCpi Vector Series(Product No.:RE-0017～RE-0024)

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