Cat. # 9053

For Research Use

TaKaRa

E. coli CJ236 Competent Cells

Product Manual

v202012Da

Table of Contents

I. Description

Competent Cells are prepared by Hanahan's method modified by Takara Bio and have a transformation efficiency of 1 x 10^7 cfu/μg when 100 μl of the cells are transformed by 1 ng pUC119.
As *E. coli* CJ236 Competent Cells can be used as a host for preparation of ssDNA with a part of Thymine (T) in its DNA replaced with deoxyuracil (dU), this product is essential for site-directed mutagenesis with Kunkel method. The ssDNA collected after transformation or transduction using this product can be available for mutagenesis with Kunkel method.

II. Components

E. coli CJ236 Competent Cells	100 μl x 10
pUC119 plasmid (0.1 ng/μl)	10 μl
SOC Medium*	1 ml x 10

* SOC Medium		
	2%	Tryptone
	0.5%	Yeast extract
	10 mM	NaCl
	2.5 mM	KCl
	10 mM	$MgSO_4$
	10 mM	$MgCl_2$
	20 mM	Glucose

III. Storage

-80℃

Note: If it is not stroed at -80℃, the transformation efficiency may decrease. In this case, it is recommended to confirm the efficiency by using supplied pUC119 prior to use an application. Never store this product in liquid nitrogen.

IV. Protocol

A. Transformation with a plasmid vector

(1) Thaw *E. coli* CJ236 Competent Cells on ice just before use.
(2) Gently mix cells and transfer 100 μl into a 14 ml round-bottom tube (CORNING #352059 or #352057)
Note : Do not use a vortex to mix cells.
(3) Add DNA sample (10 ng or less is recommended).
(4) Keep in the ice bath for 30 min.
(5) Incubte cells for 45 sec at 42℃.
(6) Return to the ice bath for 1 - 2 min.
(7) Add SOC Medium (pre-incubated at 37℃) up to a final volume of 1 ml.
(8) Incubate by shaking (160 - 225 rpm) for 1 hour at 37℃.
(9) Plate on selective media. 100 μl or less is recommended for plating on dish with φ9cm.
(10) Incubate overnight at 37℃.

B. Transduction with a M13 phage vector

(1) Follow the step (1)-(8) mentioned in A.
(2) Add 200 μl of the host (*E. coli* CJ236, A_{600}=0.8 - 1.0) into 3 ml of YT soft agar (pre-incubated at 46 - 48℃).
(3) Add a proper amount of the solution prepared at 1) into the agar, mix, and immediately spread it onto a YT-plate.
(4) Incubate at room temperature for 10 - 15 min and then, at 37℃ overnight.

【 Please read before proceeding 】

1. Place a vial of competent cells in a dry ice / EtOH bath immediately upon removal from -80℃ freezer. Keep cells in bath until you are ready to proceed.
2. Microcentrifuge tubes can be used for transformation instead of Falcon tubes (CORNING #352059 or #352057). However, the transformation efficiency may be lowered with microcentrifuge tubes.
3. It is recommended to use 10 ng or less of highly-purified DNA for transformation for 100 μl of competent cell, because of becoming inefficiency.
4. When changing an experiment scale or using a different tube, optimum condition should be considered. When using microcentrifuge tubes, incubate at 42℃ for 60 sec at the step (5) in Protocol.
5. L-broth or φb-broth can be used instead of SOC Medium. In this case, lower efficiency might be obtained.

   L-broth :

Ingredient	per liter water
Tryptone	10 g
Yeast extract	5 g
NaCl	5 g

   Adjust to around pH 7.5 with 1N NaOH and autoclave.

   φb-broth :

Ingredient	per liter water
Tryptone	20 g
Yeast extract	5 g
$MgSO_4 \cdot 7H_2O$	5 g

   Adjust to around pH 7.5 with 1N KOH and autoclave.
6. When diluting, use SOC Medium which has been added at the step A-(7).
7. Addition of Chloramphenicol (30 μg/ml) to the selective media at the step A-(9) is recommended to maintain F' plasmid in stable.
8. YT soft agar :

Ingredient	per 100 ml water
Tryptone	0.8 g
Yeast extract	0.5 g
NaCl	0.5 g

   Adjust to around pH 7.6 with 1N NaOH, add agar to the concentration of 0.6%, and autoclave.
9. YT-plate :

Ingredient	per liter water
Tryptone	8 g
Yeast extract	5 g
NaCl	5 g

   Adjust to around pH 7.6 with 1N NaOH, add agar to the concentration of 1.5%, and autoclave.
10. Host strain can be prepared by culturing competent cells.
11. It is not recommended to freeze and store the thawed competent cells. However, if necessary, freeze in a dry ice/EtOH bath and return to -80℃. The transformation efficiency can be lowered by more than one magnitude.

V. Quality

1 ng of pUC119 was transformed and selected by Amp^+ selective media plating.
Transformation efficiency : >1 x 10^7 cfu / μg pUC119

VI. Genotype

E. coli CJ236 : *dut*1, *ung*1, *thi*-1, *recA*1 / pCJ105 (F' *cam*^{r})

VII. Cell density

1 - 2 x 10^9 bacteria/ml

VIII. References

1) Hanahan D. *J Mol Biol*. (1983) **166**: 557.
2) Kunkel T A. *Proc Natl Acad Sci USA*. (1985) **82**: 488.
3) Kunkel T A. *Methods in Enzymology.* (1985) **154**: 367.
4) Zoller M J and Smith M. *Methods in Enzymology.* (1983) **100**: 468.