Cat. # 9055

For Research Use

TaKaRa

E. coli MV1184 Competent Cells

Product Manual

v201410Da

I. Components

E. coli MV1184 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

II. Specification

E. coli MV1184 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 with 1 ng of pUC119. As *E. coli* MV1184 is an amber suppressor(-) strain in which only non-amber DNA vectors can propagate, this product can be used for selecting amber-mutated DNA. As containing F' plasmid, it can be also used as a host of M13 phage vectors or phagemid vectors in preparation of ssDNA. When transformation of pUC vectors or transduction of M13 phage vector DNAs, recombinants can be selected easily by adding X-Gal and IPTG to a media utilizing α-complementarity to β-galactosidase of the Competent Cells.

X-Gal : 5-Bromo-4-Chloro-3-Indolyl-β-D-Galactoside
IPTG : Isopropyl-β-D-thiogalactopyranoside

III. Protocols

A. Transformation with a plasmid vector

1) Thaw *E. coli* MV1184 Competent Cells on ice bath just before use.
2) Gently mix cells and transfer 100 μl of Competent Cells into a 14 ml round-bottom tube.
   Important : Do not use a vortex to mix cells.
3) Add DNA sample (≦ 10 ng 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 9 cm diameter dish.
10) Incubate overnight at 37℃.

B. Transduction with a M13 phage vector

1) Follow step 1) - 8) of III-A.
2) Add 200 μl of the host cells (*E. coli* MV1184, 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 step 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.

IV. Precautions for Use

1) Place a vial of the 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) You may use 1.5 ml microcentrifuge tubes instead of 14 ml round-bottom tubes (BD company Code: 352059 or 352057, etc.) for transformation, but it may reduce efficiency.
3) When using 100 μl of Competent Cells, apply 10 ng or less of highly purified plasmid DNA. If not, transformation efficiency might decrease.
4) When changing an experiment scale, optimum condition should be considered.
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 1 L water
Bacto tryptone	10 g
Bacto yeast extract	5 g
NaCl	5 g

   Adjust to around pH7.5 with 1 N NaOH and autoclave.

   • φb-broth:

Ingredient	per 1 L water
Bacto tryptone	20 g
Bacto yeast extract	5 g
$MgSO_4 \cdot 7H_2O$	5 g

   Adjust to around pH7.5 with 1 N KOH and autoclave.

6) Medium contents

   YT soft agar:

Ingredient	per 100 ml water
Bacto tryptone	0.8 g
Bacto yeast extract	0.5 g
NaCl	0.5 g

   Adjust to around pH7.6 with 1 N NaOH, add agar to the concentration of 0.6%, and autoclave.

   YT-plate:

Ingredient	per liter
Bacto tryptone	8 g
Bacto yeast extract	5 g
NaCl	5 g

   Adjust to pH7.6 with 1 N NaOH, add agar to the concentration of 1.5%, and autoclave.

7) *E. coli* MV1184 cells can be prepared by culturing Competent Cells.
8) When adding X-Gal or IPTG, follow the procedures described as below:
   • Add 100 mM IPTG to be 100 μl/100 ml agar medium and 25 μl/3 ml soft agar.
   • Add 20 mg X-Gal/ml dimethylhormeamide to be 200 μl/100 ml medium and 50 μl/3 ml soft agar.
9) It is not recommended to refreeze 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. Transformation efficiency

When 100 μl of *E. coli* MV1184 Competent Cells were transformed with 1 ng of pUC119 according to III-A and the transformants were selected on a LB-agar plate containing Ampicilin, the transformation efficiency was > 1.0×10^7 cfu/μg pUC119.

VI. Stability of F' plasmid

The white colonies were less than 1% of the total colonies when *E. coli* MV1184 Competent Cells were transformed with pUC119 and plated on a LB-agar plate containing Ampicillin (100 μg/ml), IPTG (0.3 mM), and X-Gal (60 μg/ml).

VII. Genotype

E. coli MV1184 : *ara,* Δ (*lac-proAB*), *rpsL*, *thi* (φ80 *lacZ*′ Δ M15), Δ (*srl-recA*) 306: : *Tn10* (*tet*^{r})/F′ [*traD36*, *proAB*$^+$, *lac I*^{q}, *lacZ*′ Δ M15]

VIII. Cell density

1 - 2 X 10^9 bacteria/ml

IX. Storage

-80℃

Note: If it is not stored at -80℃ , transformation efficiency may decrease. In this case, it is recommended to confirm the efficiency by using supplied pUC119 prior to use an application.
Do not store in liquid nitrogen.

X. Reference

1) Hanahan, D. (1983) *J. Mol. Biol.* **166**, 557.

XI. Related product

E. coli MV1184 Electro-Cells (Cat. #9025)
pUC118 DNA (Cat. #3318)
pUC119 DNA (Cat. #3319)
pUC118 DNA/BAP (Cat. #3320 - 3324)
pTV118N DNA (Cat. #3328)
X-Gal (5-Bromo-4-Chloro-3-Indolyl-β-D-Galactoside) (Cat. #9031)
IPTG (Isopropyl-β-D-thiogalactopyranoside) (Cat. #9030)