invitrogen™

MAX Efficiency® DH10Bac™ Competent Cells

Cat. No. 10361-012

Size: 0.5 ml
Store at -70°C.
Do not store in liquid nitrogen.

Information for European Customers: These cells are genetically modified and carry the pBR322-derived plasmid pMON7124 (*bom*$^+$, *tra*$^-$, *mob*$^-$). As a condition of sale, this product must be used in accordance with all applicable local legislation and guidelines including EC Directive 90/219/EEC on the contained use of genetically modified organisms.

Description: MAX Efficiency® DH10Bac™ Competent Cells have been prepared using a patented modification of the procedure of Hanahan (1). MAX Efficiency® DH10Bac™ Competent Cells are used to produce recombinant baculovirus molecules for the expression of eukaryotic proteins (2). MAX Efficiency® DH10Bac™ Competent cells contain the parent bacmid bMON14272 and the helper plasmid pMON7124. The parent bacmid contains a mini-F replicon, the kanamycin resistance gene, an attTn7 site and the *lac*Zα complementation factor. The helper plasmid contains the *tns*ABCD region which supplies the transposition proteins required for insertion of the mini-Tn7 from the donor plasmid into its target site on the parent bacmid. The donor pFastBac™ plasmid carries a Tn7 element containing the gentamicin resitatnce gene, the baculovirus polyhedrin promoter, a multiple cloning site region and the SV40 poolyadenylation signal. A composite bacmid molecule is produced when MAX Efficiency® DH10Bac™ Competent Cells are transformed with a donor pFastBac™ plasmid containing a coding sequence cloned into the multiple cloning site (the composite appears as a white colony on the selective medium described in the transformation procedures). The composite bacmid can then be isolated and transfected into insect cells, resulting in the production of infectious recombinant baculovirus particles.

MAX Efficiency® DH10Bac™ Competent Cells are resistant to the effects of ligase and ligase buffer and can tolerate the addition of small amounts of undiluted ligation reactions (see Note 3). The ϕ80d*lac*ZDM15 marker provides α-complementation of the β-galactosidase gene from the bacmid vector and therefore can be used for blue/white screening of colonies on bacterial plates containing X-gal.

Part no. 10361012.pps Rev date.: 25 October 2006

Genotype

F$^-$ *mcr*A Δ(*mrr-hsd*RMS-*mcr*BC) ϕ80*lac*ZΔM15 Δ*lac*X74 *rec*A1 *end*A1 *ara*D139 Δ (*ara*, *leu*)7697 *gal*U *gal*K λ$^-$ *rps*L *nup*G /pMON14272 / pMON7124

Component	Amount Per Vial
DH10Bac™ Competent Cells	100 µl
pUC19 DNA (0.01 µg/ml)	100 µl

Quality Control: MAX Efficiency® DH10Bac™ Competent Cells consistently yield $> 1 \times 10^8$ transformants/µg pUC19 with non-saturating amounts (50 pg) of DNA. Saturating amounts of pUC19 (25 ng) generate $> 1 \times 10^5$ ampicillin-resistant colonies in a 100-µl reaction. A transposition frequency of > 8% (% white colonies) is obtained with 1 ng of pFastBac-gus.

Transformation Procedure: A stock pUC19 solution (0.01 µg/ml) is provided as a control to determine the transformation efficiency. The stock solution of pFastBac-gus (0.2 µg/ml), provided with pFastBac™1 Expression Vector (Cat. No. 10360-014), can be used as a control for the transposition frequency. To obtain maximum transformation efficiency, the experimental DNA must be free of phenol, ethanol, protein and detergents.

1. Thaw competent cells on wet ice. Place required number of 17 × 100 mm polypropylene tubes (Falcon® 2059) on ice.
2. Gently mix cells, then aliquot 100 µl of competent cells into chilled polypropylene tubes.
3. Refreeze any unused cells in the dry ice/ethanol bath for 5 minutes before returning to the -70°C freezer. Do not use liquid nitrogen.
4. To determine the transformation efficiency, add 5 µl (50 pg) pUC19 control DNA to one tube containing 100 µl competent cells. To determine the transposition efficiency, add 5 µl (1 ng) pFastBac-gus control DNA to 100 µl of competent cells. Move the pipette through the cells while dispensing. Gently tap tube to mix.
5. Incubate cells on ice to 30 minutes.
6. Heat-shock cells 45 seconds in a 42°C water bath; do not shake.
7. Place on ice for 2 minutes.
8. Add 0.9 ml room temperature S.O.C. Medium (Cat. No.. 15544-034).
9. **For tubes with pFastBac™ constructs**: Shake at 225 rpm (37°C) for 4 hours.
   For tubes with pUC19 control DNA: Shake at 225 rpm (37°C) for 1 hour.

10. Dilute the reaction containing the pFastBac-gus control DNA 1:10, 1:100, and 1:1000 with S.O.C. Medium. Spread 100 µl of this dilution on Luria Agar (Miller's LB Agar) plates that contain kanamycin sulfate, tetracycline, gentamicin, X-gal and IPTG. See Note 4.
11. Dilute the transformations with the pUC19 control DNA 1:100 and plate on LB agar with 100 µg/ml ampicillin.
12. Dilute experimental reactions as necessary and spread 100 to 200 µl of this dilution as described in Step 10.
13. Incubate for no less than 24 hours at 37°C. See notes 6 and 7.

Growth of Transformants for Plasmid Preparations:

MAX Efficiency® DH10Bac™ Cells which have been transformed with pFastBac™-based plasmid should be grown at 37°C overnight in LB broth containing 50 µg/ml kanamycin sulfate, 7 µg/ml gentamicin and 10 µg/ml tetracycline. A 1.5-ml overnight culture inoculated from a single colony will yield sufficient amounts of composite bacmid DNA for several insect cell transfections.

Notes:

1. For best results, each vial of cells should be thawed only once. Although the cells are refreezable, subsequent freeze-thaw cycles will lower transformation frequencies by approximately two-fold.
2. Media other than S.O.C. Medium can be used, but the transformation efficiency will be reduced. Expression in Luria Broth reduces transformation efficiency a minimum of two- to three-fold (3).
3. Transformation efficiencies will be approximately 10-fold lower for ligation of inserts to vectors than for an intact control plasmid. DH10Bac™ cells can tolerate the addition of up to 1 µl (5 to 50 ng) of an undiluted ligation reaction without a significant loss is transformation efficiency. We have observed that the cells begin to saturate with 10 to 50 ng of DNA (4).
4. Other media may be used for plates; however, the color intensity of the blue/white selection will be reduced.

5. Neither incubation of the plates at a higher temperature nor storage of the plates at 4°C after the 24-hour growth period produces an increase in color intensity.
6. Different size colonies are to be expected. True white colonies (containing composite bacmids) tend to be larger in size. Select the largest, most isolated colonies to avoid cross-contamination.
7. The plates must be incubated at 37°C for not less that 24 hours. Shorter incubation times can result in difficulties interpreting the blue/white selection.
8. Transformation efficiency (CFU/µg):

$$\frac{\text{CFU in control plate}}{\text{pg pUC 19 used in transformation}} \times \frac{1 \times 10^6 \text{ pg}}{\mu\text{g}} \times \text{dilution factor(s)}$$

For example, if 50 pg pUC yields 100 colonies when 100 µl of a 1: 10 dilution is plated, then:

$$\text{CFU}/\mu\text{g} = \frac{100 \text{ CFU}}{50 \text{ pg}} \times \frac{1 \times 10^6 \text{ pg}}{\mu\text{g}} \times \frac{1 \text{ ml}}{0.1 \text{ ml plated}} \times 10 = 2 \times 10^8$$

References:

1. Hanahan, D. (1983) *J. Mol. Biol. 166*, 557.
2. Luckow, V.A., Lee, S.C., Barry, G.F., and Olins, P.O. (1993) *J. Virol. 67:*4566.
3. Jessee, J. (1988) *Focus® 10:3*, 53.
4. Jessee, J. (1984) *Focus® 6:4*, 5.

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