invitrogen

MAX Efficiency® DH5αF'IQ™ Competent Cells

Cat. No. 18288-019 **Size: 1 ml**
Store at -70°C
Do not store in liquid nitrogen

Description

MAX Efficiency® DH5αF'IQ™ Competent Cells have been prepared by a patented modification of the procedure of Hanahan (1). DH5αF'IQ™ contains an F' episome and is a host for the M13mp cloning vectors as well as plasmid-derived vectors. DH5αF'IQ™ lawn cells are provided to allow for plaque formation with M13mp vectors.

Genotype

F⁻ϕ80*lac*ZΔM15 Δ(*lac*ZYA-*arg*F) U169 *rec*A1 *end*A1 *hsd*R17 (r_k-, m_k+) *pho*A *sup*E44 λ⁻ *thi*⁻1 *gyr*A96 *rel*A1/F´ *pro*AB+ *lac*IqZΔM15 zzf::Tn5 [KmR].

Component	Amount per Vial
DH5αF'IQ™ Competent Cells	200 µl
DH5αF'IQ™ Lawn Cells	1500 µl
M13mp19 RF DNA (0.01 µg/ml)	10 µl

Quality Control

MAX Efficiency® DH5αF'IQ™ Competent Cells consistently yield >3 × 10^8 colony forming units/µg pUC19 with non-saturating amounts (50 pg) of DNA. The cells yield >1 × 10^8 plaque forming units/µg M13mp19 RF with non-saturating amounts (5 pg) of DNA. Saturating amounts of M13mp19 RF (25 ng) generate >1 x 10^6 plaque forming units in a 100-µl reaction.

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Transformation Procedure

A stock M13mp19 RF solution (0.01 µg/ml) is provided as a control to determine the transformation efficiency. 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 competent cells into chilled polypropylene tubes.
3. Refreeze any unused cells in the dry ice/ethanol bath for 5 minutes before returning them to the -70°C freezer. Do not use liquid nitrogen.
4. To determine transformation efficiency, add 5 µl of a 1:10 dilution (5 pg) of control DNA to one tube containing 100 µl competent cells. Move the pipette through the cells while dispensing. Gently tap tube to mix.
5. For DNA from ligation reactions, dilute the reactions 5-fold in 10 mM Tris-HCl (pH 7.5), 1 mM EDTA. Add 1 µl of the dilution to the cells (1-10 ng DNA), moving the pipette through the cells while dispensing. Gently tap tubes to mix.
6. Incubate cells on ice for 30 minutes.
7. Heat-shock cells 45 seconds in a 42°C water bath; do not shake.
8. Place on ice for 2 minutes.
9. Add 0.9 ml of room temperature S.O.C. Medium (Cat. No. 15544-034).

10. Add a 20- to 200-µl aliquot of transformed cells to 3.0 ml YT top agar. Prepare top agar aliquots beforehand by tempering to 45°C and adding 50 µl 2% X-gal (Cat. No. 15520-034) or Bluo-gal and 10 µl 100 mM IPTG (Cat. No. 15529-019). Mix well, then add 100 µl lawn cells to each aliquot.
11. Plate on YT plates. Pour overlay gently onto plate and spread evenly. Allow top agar to solidify. Invert plates and incubate at 37°C overnight.
12. The remaining lawn cells may be refrozen by placing in a dry ice/ethanol bath or by returning directly to -70°C freezer.

Calculating Transformation Efficiency (PFU/µg)

$$\frac{\text{PFU in control plate}}{\text{pg M13mp19 used in transformation}} \times \frac{1 \times 10^6 \text{ pg}}{\text{µg}} \times \text{dilution factor}$$

For example, if 5 pg M13mp19 RF yields 200 plaques when 100 µl of the 1 ml reaction is plated, then:

$$\text{PFU/µg} = \frac{200 \text{ PFU}}{5 \text{ pg}} \times \frac{1 \times 10^6 \text{ pg}}{\text{µg}} \times 10 = 4 \times 10^8 \text{ PFU/µg}$$

References

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

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