Invitrogen
life technologies

ElectroMAX™ *Agrobacterium tumefaciens* LBA4404 Cells

Cat. No. 18313-015 **Size: 0.2 ml**
Store at -80°C
(Do not store in liquid nitrogen)

Description

ElectroMAX™ *A. tumefaciens* LBA4404 Cells are *Agrobacterium tumefaciens* cells which can be transformed by electroporation (1,2,3). These cells can only be transformed by electroporation and are **not** transformable by "heat shock". *A. tumefaciens* LBA4404 cells contain the disarmed Ti plasmid pAL 4404 which has only the *vir* and *ori* region of the Ti plasmid (4). Historically, the recombinant T-DNAs have been introduced into *A. tumefaciens* cells by using electroporation or triparental mating. These recombinant DNAs are then able to migrate from *A. tumefaciens* cells into plant cells using components provided by the plasmid pAL 4404 (5,6). Electroporating *A. tumefaciens* LBA4404 cells with a binary vector like pBI121 involves fewer steps and less time than the triparental mating procedure for obtaining transformants. In addition, the triparental mating procedure involves the risk of *E. coli* contamination of the *A. tumefaciens* transformants, a problem which is eliminated by using electroporation (3).

Component	Amount
ElectroMAX™ LBA4404 Cells	5 x 40 µl
pBI121 (1 ng/µl)	10 µl

Quality Control

ElectroMax™ *A. tumefaciens* LBA4404 Cells are tested for transformation efficiency using the protocol on the next page and the following electroporator conditions: 2.0 kV, 200 Ω, 25 µF. Transformation efficiency should be > 5.0 x 10^6 transformants per µg pBI121.

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For technical questions about this product, call the Invitrogen Tech-Line℠ U.S.A. 800 955 6288

Transformation Procedure

A stock pBI121 DNA solution (1 ng/µl) is provided as a control to determine the transformation efficiency. To obtain maximum transformation efficiency, the experimental DNA must be free of phenol, ethanol, salts, protein and detergents.

1. Prepare YM Medium (see Note 1). A recipe is provided on the next page.
2. Add DNA to microcentrifuge tubes.
   A. To determine transformation efficiency, add 1 µl of the pBI121 control DNA to a microcentrifuge tube.
   B. For plasmid DNA from minipreps, precipitate the reactions with ethanol and resuspend in 10 mM Tris-HCl (pH 7.5) and 1 mM EDTA. The concentration of resuspended DNA should not exceed 100 ng/µl. Add 1 µl of the DNA to a microcentrifuge tube.
3. Thaw the ElectroMAX™ *A. tumefaciens* LBA4404 Cells on wet ice.
4. When cells are thawed, mix cells by tapping gently. Add 20 µl of cells to each chilled microcentrifuge tube containing DNA.
5. Refreeze any unused cells in a dry ice/ethanol bath for 5 minutes before returning them to the -80°C freezer. Do not use liquid nitrogen.
6. Pipet the cell/DNA mixture into a chilled 0.1 cm cuvette and electroporate. If you are using the BTX® ECM® 630 electroporator, we recommend using the following electroporator conditions: 2.0 kV, 200 Ω, 25 µF (see Note 2).
7. To the cells in the cuvette, add 1.0 ml of room temperature YM Medium and transfer the solution to a 15 ml snap-cap tube (*e.g.* Falcon™ tube).
8. Shake at 225 rpm (30°C) for 3 hours.

9. Dilute cells transformed with the control pBI121 DNA 1:10 with YM Medium. Spread 100 µl of this dilution on prewarmed YM plates containing 100 µg/ml streptomycin and 50 µg/ml kanamycin.
10. Dilute experimental reactions as necessary and spread 100-200 µl of this dilution on prewarmed selective plates.
11. Incubate plates for 48-56 hours at 30°C.

Formulation of YM Medium (7)

Component	Final Conc. of Component	Amount/1.0 L
Yeast extract	0.04%	0.4 g
Mannitol	1.0%	10.0 g
NaCl	1.7 mM	0.1 g
$MgSO_4 \cdot 7H_2O$	0.8 mM	0.2 g
$K_2HPO_4 \cdot 3H_2O$	2.2 mM	0.5 g

Dissolve the components in 900 ml distilled water, and adjust to pH 7.0. Bring volume to 1 L with distilled water. Autoclave the medium at 121°C, 15 psi for 20 min. Cool to room temperature before use.

Notes

1. For best results, use YM Medium for expression and plating. Use of other media such as LB broth or LB broth with mannitol will result in 2 to 5 fold lower transformation efficiency as well as smaller colonies on plates.
2. If you are using an electroporator other than the BTX® ECM® 630 electroporator, you may need to vary the setting to achieve optimal transformation efficiency.

3. Transformation efficiency (CFU/µg):

$$\frac{\text{CFU in control plate}}{\text{pg pBI121}} \times \frac{1 \times 10^3 \text{ ng}}{\mu\text{g}} \times \frac{\text{volume of transformants}}{\text{volume plated}} \times \text{dilution factor}$$

For example, if 1 ng of pBI121 yields 50 colonies when 100 µl of a 1:10 dilution is plated, then:

$$\text{CFU}/\mu\text{g} = \frac{50 \text{ CFU}}{1 \text{ ng}} \times \frac{1 \times 10^3 \text{ ng}}{\mu\text{g}} \times \frac{1 \text{ ml}}{0.1 \text{ ml plated}} \times 10 = 5.0 \times 10^6$$

References

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2. Cangelosi, G., Best, E., Martinetti, G. and Nester, E. (1991) *Methods in Enzymol. 204,* 384.
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7. Vincent, J. A manual for the practical study of root-nodule bacteria. (1985) *IBP Handbook No. 15*.