

High Efficiency LBA4404 ElectroComp Agrobacterium

Description

Intact Genomics LBA4404 ElectroComp Agrobacterium cells are optimized for the highest transformation efficiencies which is ideal for applications requiring high transformation efficiencies, such as with cDNA or gDNA library construction. The LBA4404 strain is useful for transgenic operations of tomatoes, tobacco and other plants. LBA4404 contains a rifampicin resistance gene (rif) and also has streptomycin resistance. LBA4404 strain also contains a octoprine-type Ti plasmid pAL4404 without self-transport function, which contains the vir gene.

Specifications

Competent cell type:	Electrocompetent
Species:	<i>A. tumefaciens</i>
Strain:	LBA4404
Format:	Tubes
Transformation efficiency:	$\geq 1 \times 10^7$ cfu/ μ g pCAMBIA1391z DNA
Blue/white screening:	No
Shipping condition:	Dry ice

Genotype

Ach5 (rif^r) Ti pAL4404 (strepr) Octopine

Reagents Needed for One Reaction

LBA4404 ElectroComp Agrobacterium:	25 μ l
DNA (pCAMBIA1391z, 100 pg/ μ l):	1 μ l
Recovery medium:	1 ml

Storage

LBA4404 ElectroComp Agrobacterium:	-80 °C
pCAMBIA1391z control DNA:	-20 °C
Recovery medium:	4 °C

Quality Control

Transformation efficiency is tested by using the pCAMBIA1391z control DNA supplied with the kit and using the protocol in this manual. Transformation efficiency should be $\geq 1 \times 10^7$ CFU/ μ g pCAMBIA1391z DNA. Untransformed cells are tested for appropriate antibiotic sensitivity.

General Guidelines

Follow these guidelines when using LBA4404 ElectroComp Agrobacterium:

- Handle competent cells gently as they are highly sensitive to changes in temperature or mechanical lysis caused by pipetting.
- Thaw competent cells on ice, and transform cells immediately following thawing. After adding DNA, mix by tapping the tube gently. Do not mix cells by pipetting or vortexing.

Note: A high-voltage electroporation apparatus such as Bio-Rad Gene Pulser II #165-2105, capable of generating field strengths of 16 kV/cm is required.

Calculation of Transformation Efficiency

Transformation Efficiency (TE) is defined as the number of colony forming units (cfu) produced by transforming 1 μ g of plasmid into a given volume of competent cells.

$$TE = \text{Colonies}/\mu\text{g}/\text{Plated}$$

Transform 1 μ l of (500 pg/ μ l) pCAMBIA1391z control plasmid into 25 μ l of cells, add 974 μ l of Recovery Medium. Recover for 3 hours and plate 100 μ l. Count the colonies on the plate in two days. If you count 500 colonies, the TE is calculated as follows:

$$\begin{aligned}\text{Colonies} &= 500 \\ \mu\text{g of DNA} &= 0.0005 \\ \text{Dilution} &= 100/1000 = 0.1 \\ \text{TE} &= 500/.0005/.1 = 1 \times 10^7\end{aligned}$$

Transformation Protocol

Use this procedure to transform LBA4404 ElectroComp Agrobacterium. Do not use these cells for chemically transformation.

- 1) Place sterile cuvettes and microcentrifuge tubes on ice.
- 2) Remove competent cells from the -80 °C freezer and thaw completely on wet ice (10-15 minutes).

- 3) Aliquot 1 μ l (10pg -1 μ g) of DNA to the chilled microcentrifuge tubes on ice.
- 4) When the cells are thawed, add 25 μ l of cells to each DNA tube on ice and mix gently by tapping 4-5 times. For the pCAMBIA1391z control, add 1 μ l of (500 pg/ μ l) DNA to the 25 μ l of cells on ice. Mix well by tapping. Do not pipette up and down or vortex to mix, this can harm cells and decrease transformation efficiency.
- 5) Pipette 26 μ l of the cell/DNA mixture into a chilled electroporation cuvette without introducing bubbles. Quickly flick the cuvette downward with your wrist to deposit the cells across the bottom of the well and then electroporate.
- 6) Immediately add 976 μ l of Recovery Medium or any other medium of choice to the cuvette, pipette up and down three times to re-suspend the cells. Transfer the cells and Recovery Medium to an Eppendorf tube. .
- 7) Incubate tubes at 30 °C for 3 hours at 200 RPM.
- 8) Dilute the cells as appropriate then spread 20-200 μ l cells onto a pre-warmed selective plate. For the pCAMBIA1391z control, you may plate 50 μ l of diluted transformants onto a YT plate containing 15 μ g/ml rifampicin and 50 μ g/ml kanamycin. Use sterilized spreader or autoclaved ColiRoller™ plating beads to spread evenly.
- 9) Incubate the plates for 2 - 3 days at 30 °C.

Electroporation settings

Mode	Exponential protocol
Voltage (V)	1,800 V
Capacitance	25 μ FD
Resistance	200 Ohms
Cuvette	1 mm