

# K599 ElectroCompetent Agrobacterium



|              |         |          |
|--------------|---------|----------|
| Catalog #    | 1287-12 | 1287-36  |
| Package Size | 6x50 µl | 18x50 µl |

## Description

Intact Genomics (ig®) K599 Electrocompetent Agrobacterium cells are made from a specific strain of Agrobacterium rhizogenes, Agrobacterium rhizogenes (str R) pRi2659 (agropine type). K599 was originally isolated from cucumber exhibiting hairy root disease symptoms and has been widely used for hairy root transformation(1). Agrobacterium rhizogenes is a soil-borne gram-negative bacterium that can infect most dicotyledons, a few monocotyledons and some gymnosperms. K599 Electrocompetent Agrobacterium are optimized for the highest transformation efficiencies and are useful for transgenic operations of corn, soybean, cotton, peanut, dandelion, cowpea and other plants. K599 Agrobacterium rhizogenes strain contains pRi2659 agrobacterium-type Ri plasmid and displays streptomycin resistance.

## Specifications

Competent cell type: Electrocompetent  
Species: *A. rhizogenes*  
Strain: K599  
Format: Tubes  
Transformation eff.:  $\geq 1 \times 10^5$  cfu/µg pCAMBIA1391z DNA  
Blue/white screening: No  
Shipping condition: Dry ice

## Reagents Included

- ig® K599 Electrocompetent Agrobacterium
- DNA (pCAMBIA1391z, 500 pg/µl)
- Recovery medium

## Storage

- ig® K599 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  $\geq 1 \times 10^5$  CFU/µg pCAMBIA1391z DNA. Untransformed cells are tested for appropriate antibiotic sensitivity.

## General Guidelines

Follow these guidelines when using K599 Electrocompetent 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.

## Example Calculation of Transformation Eff.

Transformation Efficiency (TE) is defined as the number of colony forming units (cfu) produced by transforming 1µg of plasmid into a given volume of competent cells.  
 $TE = \text{Colonies}/\mu\text{g}/\text{Plated}$   
Transform 1 µl of (500 pg/µl) pCAMBIA1391z control plasmid into 25 µl of cells, add 974 µl of Recovery Medium. Recover for 3 hours and plate 100 µl. 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 K599 Electrocompetent Agrobacterium. Do not use these cells for chemically transformation.

- Place sterile cuvettes and microcentrifuge tubes on ice.
- Remove competent cells from the -80 °C freezer and thaw completely on wet ice (10-15 minutes).
- Aliquot 1 µl (10pg -1 µg) of DNA to the chilled microcentrifuge tubes on ice.
- When the cells are thawed, add 25 µl of cells to each DNA tube on ice and mix gently by tapping 4-5 times. For the pCAMBIA1391z control, add 1 µl of (500 pg/µl) DNA to the 25 µl 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.
- Pipette 26 µl 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.
- Immediately add 974 µl 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. .
- Incubate tubes at 30 °C for 3 hours at 200 RPM.
- Dilute the cells as appropriate then spread 20-200 µl cells onto a pre-warmed selective plate. For the pCAMBIA1391z control, you may plate 100 µl of undiluted transformation mix onto a YT plate containing 50 µg/ml kanamycin. Use sterilized spreader or autoclaved ColiRoller™ plating beads to spread evenly.
- Incubate the plates for 2 - 3 days at 30 °C.

## Electroporation Settings

|             |                      |
|-------------|----------------------|
| Mode        | Exponential protocol |
| Voltage (V) | 1,800 V              |
| Capacitance | 25 uFD               |
| Resistance  | 200 Ohms             |
| Cuvette     | 1 mm                 |

# K599 ElectroCompetent Agrobacterium



## Related Products

- GV3101 Chem. Competent Agrobacterium (Cat.# 1082-12)
- LBA4404 Chem. Competent Agrobacterium (Cat.# 1085-12)
- EHA105 ElectroCompetent Agrobacterium (Cat.# 1284-12)
- Agrobacterium Combo Pack (Cat.# 1290-24)
- T4 DNA Ligase (Cat.# 3212)

## Technical Support

Intact Genomics is committed to supporting the worldwide scientific research community by supplying the highest quality reagents. Each new lot of our products is tested to ensure they meet the quality standards and specifications designated for the product.

Please follow the instructions carefully and contact us if additional assistance is needed. We appreciate your business and your feedback regarding the performance of our products in your applications.

## References

1. Combard, A.; Brevet, J.; Borowski, D.; Cam, K.; Tempé, J.  
Physical map of the T-DNA region of *Agrobacterium rhizogenes* strain NCPPB2659. *Plasmid* **1987**, 18, 70–75.

**Note:** All agrobacterial strains are not well studied for antibiotic resistance and there are many agrobacterial strains. Therefore, it is the customer's responsibility to make sure his/her vectors are compatible with the Agrobacterial strains if he/she uses an alternate antibiotic selection than kanamycin-selection.

