



K599 ElectroCompetent Agrobacterium

Manual

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



Important!

-80°C Storage Required

- * Immediately inspect packages
- * Freeze upon receipt



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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 Spectinomycin resistance.

Specifications:

Competent cell type: Electrocompetent

Species: *A. rhizogenes*

Strain: K599

Format: Tubes

Transformation efficiency: $\geq 1 \times 10^5$ cfu/ μ g pCAMBIA1391z DNA

Blue/white screening: No

Shipping condition: Dry ice

Product Components:

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

Storage:

- ig® K599 ElectroCompetent 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.

Calculation of Transformation Efficiency:

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

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

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

$$\text{Colonies} = 500$$

$$\mu\text{g of DNA} = 0.0005$$

$$\text{Dilution} = 100/1000 = 0.1$$

$$\text{TE} = 500/.0005/.1 = 1 \times 10^7$$

Transformation Protocol:

Use this procedure to transform K599 ElectroCompetent Agrobacterium. Do not use these cells for chemical 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 µl (10pg -1 µg) of DNA to the chilled microcentrifuge tubes on ice.
- 4) 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.
- 5) 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.
- 6) 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.
- 7) Incubate tubes at 30 °C for 3 hours at 200 RPM.
- 8) Spread 200 µl cells onto a pre-warmed selective plate, if needed, dilute the cells as appropriate then plate. For the pCAMBIA1391z control, you may plate 100 µl of undiluted transformation mix onto a fresh YT plate containing 50 µg/ml kanamycin only. You can confirm the transformants or colonies by picking and re-plating them on a YT plate containing 15 µg/ml rifampicin and 50 µg/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 uFD

Resistance: 200 Ohms

Cuvette: 1 mm

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)

Ordering Information:

- Order online within the USA. Place orders on www.intactgenomics.com using our secure Shopping Cart.
- Order by email, phone, or fax.
Email: sales@intactgenomics.com
Phone: (314) 942-3655 | Toll-free : 855-835-7172 | Fax: (314) 942-3656
- Order via our distributors.

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.

Please 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.

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