



Methionine Auxotrophic LBA4404^{Met} Electrocompetent Cells

Manual

Catalog #	1276-10	1276-30
Package Size	5x50 µl	15x50 µl



Important!

-80°C Storage Required

- * Immediately inspect packages
- * Freeze upon receipt



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Description:

Intact Genomics (ig®) Methionine-Auxotrophic LBA4404^{Met} ElectroCompetent Agrobacterium cells include modifications so that they will not grow unless free methionine is added to Minimal medium. This prevents the bacteria from overgrowing plant tissues when used for plant transformation. Methionine-Auxotrophic LBA4404^{Met} ElectroCompetent Agrobacterium cells are optimized for the highest transformation efficiencies. The LBA4404^{Met} strain is useful for transgenic operations of tomatoes, tobacco and other plants. LBA4404^{Met} contains a rifampicin resistance gene (rif). LBA4404^{Met} strain also contains an octopine-type Ti plasmid pAL4404^{Met} without self transport function, which contains the vir gene.

Benefits:

- Methionine Auxotrophic
- Enables development of more efficient transformation systems
- Reduced bacterial overgrowth during co-cultivation
- Decreased need for antibiotics

Specifications:

Competent cell type: Electrocompetent

Species: *A. tumefaciens*

Strain: LBA4404^{Met}

Format: Tubes

Transformation efficiency: $\geq 1 \times 10^7$ cfu/ μ g pCAMBIA1391z DNA

Shipping condition: Dry ice

Product Components:

- ig® Methionine-Auxotrophic LBA4404^{Met} ElectroCompetent Agrobacterium Cells
- DNA (pCAMBIA1391z, 500 pg/ μ l)
- Recovery medium

Storage:

- ig[®] Methionine Auxotrophic LBA4404^{Met} ElectroCompetent Agrobacterium Cells: -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 ig[®] Methionine-Auxotrophic LBA4404^{Met} 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 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:

$$\text{Colonies} = 500$$

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

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

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

Transformation Protocol:

Use this procedure to transform ig[®] Methionine-Auxotrophic LBA4404^{Met} 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) 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 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:

- LBA4404^{Met} Chem. Competent Agrobacterium (Cat.# 1076-05)
- EHA105^{Met} Chem/Electro Competent Agrobacterium (Cat.# 1278-10)
- GV3101 Chem. Competent Agrobacterium (Cat.# 1082-06)
- 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. Prías-Blanco, M., Chappell, T.M., Freed, E.F. et al. An Agrobacterium strain auxotrophic for methionine is useful for switchgrass transformation. *Transgenic Res* 31, 661–676 (2022). <https://doi.org/10.1007/s11248-022-00328-4>

Additional Information:

The auxotrophic *Agrobacterium tumefaciens* strains were developed with funding from the U.S. Department of Energy's Center for Bioenergy Innovation (CBI).

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