



ig® Thymidine-Auxotrophic EHA101^{Thy} Agrobacterium ElectroCompetent Cells

Manual

Catalog #	1402-10	1402-30
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®) Thymidine-Auxotrophic EHA101^{Thy} Agrobacterium ElectroCompetent Cells include modifications so that they will not grow unless 50 mg/L of thymidine is added to Minimal medium. This prevents the bacteria from overgrowing plant tissues when used for plant transformation. Thymidine-Auxotrophic EHA101^{Thy} Agrobacterium ElectroCompetent Cells are optimized for the highest transformation efficiencies. The EHA101^{Thy} strain is useful for transgenic operations of rice, tobacco and other plants. EHA101^{Thy}- thyA knockout mutant, kanamycin-resistant, derived from EHA101, itself a derivative of A281 (A136/pTiBo542).

Benefits:

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

Specifications:

Competent cell type: Electroporation Competent

Species: *A. tumefaciens*

Strain: EHA101^{Thy}

Format: Tubes

Transformation efficiency: $\geq 1 \times 10^3$ cfu/ μ g pIG7spe DNA

Shipping condition: Dry ice

Product Components:

- ig® Thymidine-Auxotrophic EHA101^{Thy} Agrobacterium ElectroCompetent Cells
- DNA (pIG7spe, 500 pg/ μ l)
- Recovery medium*

* Client needs to add 50 mg/L of thymidine (not provided) to recovery media before use

Storage:

- ig® EHA101^{Thy} Agrobacterium ElectroCompetent Cells: -80 °C
- pIG7spe control DNA: -20 °C
- Recovery medium: 4 °C

Transformation Protocol:

Use this procedure to transform ig® Thymidine-Auxotrophic EHA101^{Thy} Agrobacterium ElectroCompetent Cells. 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 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 pIG7spe 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 with 50 mg/L of thymidine 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 a 15ml of culture tube.
- 7) Incubate tubes at 30 °C for 3 hours at 200 RPM.
- 8) Spread 20-200 µl cells onto a pre-warmed selective plate. For the pIG7spe control DNA, you may plate 100 µl of undiluted transformation mix onto a YT plate containing 50 µgTM/ml of thymidine, 15 µg/ml rifampicin and 50 µg/ml spectinomycin. 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

Quality Control:

Transformation efficiency is tested by using the pIG7spe control DNA supplied with the kit and using the protocol in this manual. Transformation efficiency should be $\geq 1 \times 10^3$ CFU/ μ g pIG7spe DNA. Untransformed cells are tested for appropriate antibiotic sensitivity.

General Guidelines:

Follow these guidelines when using ig® Thymidine-Auxotrophic EHA101^{Thy} Agrobacterium ElectroCompetent Cells:

- 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) pIG7spe 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 50 colonies, the TE is calculated as follows:

$$\text{Colonies} = 50$$

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

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

$$TE = 50/.0005 = 1 \times 10^6$$

Related Products:

- EHA101^{Thy} Chemically Competent Agrobacterium (Cat.# 1302)
- EHA105^{Met} Chem/Electro Competent Agrobacterium (Cat.# 1078, 1278)
- GV3101 Chemically Competent Agrobacterium (Cat.# 1082)
- EHA105 ElectroCompetent Agrobacterium (Cat.# 1284)
- Agrobacterium ElectroCompetent Combo Pack (Cat.# 1290)
- 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:

Aliu E., Azanu MK., Wang K., Lee K. Generation of thymidine auxotrophic *Agrobacterium tumefaciens* strains for plant transformation
<https://www.biorxiv.org/content/10.1101/2020.08.21.261941v1.full.pdf>

Additional Information:

The auxotrophic *Agrobacterium tumefaciens* strains were developed with support by National Science Foundation Plant Genome Research Program Grant 1725122 and 1917138 to K.W., by the Iowa State University Interdepartmental Plant Biology Major fellowship to EA and MA, by the USDA NIFA Hatch project #IOW04341 and by State of Iowa funds and by the Crop Bioengineering Center of Iowa State University.

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Our hours are Monday - Friday, 8AM to 5PM, U.S. central standard time (CST).

Intact Genomics, Inc.

11840 Westline Industrial Drive, Suite 120,
St. Louis, MO. 63146, USA

Phone: (314) 942-3655 | **Toll-free :** 855-835-7172 | **Fax:** (314) 942-3656

Email: sales@intactgenomics.com | ig@intactgenomics.com

<http://www.intactgenomics.com>

