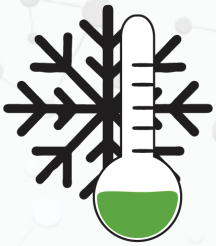
ig® Thymidine-Auxotrophic EHA101^{Thy} Agrobacterium Chemically Competent Cells

Manual

Catalog #	1302-05	1302-15
Package Size	5x50 µl	15x50 µl



Important!

-80°C Storage Required

* Immediately inspect packages
* Freeze upon receipt

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Intact Genomics, Inc.

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Description

Intact Genomics (ig®) Thymidine-Auxotrophic EHA101^{Thy} Agrobacterium Chemically Competent 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} Chemically Competent Agrobacterium cells are optimized for the highest transformation efficiencies. The EHA101^{Thy} strain is useful for transgenic operations of rice, tobacco and other plants. EAH101^{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: Chemically Competent

Species: A. tumefaciens

Strain: EHA101^{Thy}

Format: Tubes

Transformation efficiency: ≥ 1 x 10^3 cfu/µg pIG7spe DNA

Shipping condition: Dry ice

Product Components

- ig® Thymidine-Auxotrophic EHA101^{Thy} Chemically Competent Agrobacterium Cells
- DNA (pIG7spe, 500 pg/µl)
- Recovery medium*

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

Storage

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

Transformation Protocol

Use this procedure to transform ig® Thymidine-Auxotrophic EHA101^{Thy} Chemically Competent Agrobacterium Cells.

1) Place 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 (10 pg -1 µg) of DNA to the chilled microcentrifuge tubes on ice.
4) When the cells are thawed, add 50µl 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 50 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) Keep tubes on ice for 5 minutes, and then transfer to liquid nitrogen or dry ice for 5 minutes.
6) Incubate tubes for additional 5 minutes in 37°C water bath.
7) Immediately add 949 µl of Recovery Medium or any other medium of choice to the cuvette, pipette up an d down three times to re-suspend the cells. Transfer the cells and Recovery Medium to a 15ml culture tube.
8) Incubate tubes at 30 °C for 3 hours at 200 RPM.
9) Spread 20-200 µl cells onto a pre-warmed selective plate. For the pIG7spe control, you may plate 100 µl of undiluted transformation mix onto a YT plate containing 50 µg/ml™ of thymidine, 15 µg/ml rifampicin and 50 µg/ml spectinomycin. Use sterilized spreader or autoclaved ColiRoller plating beads to spread evenly.
10) Incubate the plates for 2 - 3 days at 30 °C.

Note: Liquid nitrogen or dry ice is required but not provided, as well as 37^{o}C water bath is required but not provided.

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 ≥1 x 10^3 CFU/µg pIG7spe DNA. Untransformed cells are tested for appropriate antibiotic sensitivity.

General Guidelines:

Follow these guidelines when using ig® Thymidine-Auxotrophic EHA101^{Thy} Agrobacterium Chemically Competent 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 1µg of plasmid into a given volume of competent cells.

TE = Colonies/µg/Plated

Transform 1 µl of (500 pg/µl) pIG7spe 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 50 colonies, the TE is calculated as follows:

Colonies = 50

µg of DNA = 0.0005

Dilution = 100/1000 = 0.1

TE = 50/.0005= 1x10^6

Related Products

- EHA101^{Thy} ElectroCompetent Agrobacterium (Cat.# 1402)
- EHA105^{Met} Chem/Electro Competent Agrobacterium (Cat.# 1078, 1278)
- GV3101 Chem. Competent Agrobacterium (Cat.# 1082)
- EHA105 ElectroCompetent Agrobacterium(Cat.# 1284)
- Agrobacterium 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.

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