EHA105 (pSoup) ElectroCompetent Agrobaterium

Manual

Catalog #	1284PS-12	1284PS-36
Package Size	6x50µl	18x50µl

Important!

-80°C Storage Required

* Immediately inspect packages
* Freeze upon receipt

visit us online for more products & custom services

Intact Genomics, Inc.

Table of Contents

Description:

Intact Genomics (ig®) EHA105 (pSoup) ElectroCompetent Agrobacterium cells are optimized for the highest transformation efficiencies ideal for applications with cDNA or gDNA library construction. EHA105 (pSoup) contains a rifampicin resistance gene (rif). EHA105 (pSoup) strain also contains an amber basic Ti plasmid pEHA105 (pTiBo542DT-DNA), which contains the vir gene. The pSoup plasmid aids in replication of pGreen, 62SK, and pGs2 series plasmids. This system is used for Agrobacterium-mediated transformation of dicots such as Arabidopsis thaliana, tobacco, potatoes, and monocots like corn.

Specifications:

Competent cell type: Electrocompetent

Species: *A. tumefaciens*

Strain: EHA105 (pSoup)

Format: Tubes

Transformation efficiency: ≥ 1 x 10^7 cfu/µg pCAMBIA1391z DNA

Shipping condition: Dry ice

Reagents Included:

- ig® EHA105 (pSoup) Electrocompetent Agrobacterium
- DNA (pCAMBIA1391z, 500 pg/µl)
- Recovery medium

Genotype:

C58 (rif^R), Ti pEHA105 (pTiBo542DT-DNA), pSOUP (tet^R), Succinamopine

Storage:

- ig® EHA105 (pSoup) ElectroComp. Agro. Cells: -80ºC
- pCAMBIA1391z control DNA: -20 ºC
- Recovery medium: 4 ºC

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 ≥1 x 10^7 CFU/µg pCAMBIA1391z DNA. Untransformed cells are tested for appropriate antibiotic sensitivity.

General Guidelines:

Follow these guidelines when using EHA105 (pSoup) 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 1µg of plasmid into a given volume of competent cells.

TE = Colonies/µg/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:

Colonies = 500

µg of DNA = 0.0005

Dilution = 100/1000 = 0.1

TE = 500/.0005/.1 = $1x10^7$

Transformation Protocol:

Use this procedure to transform ig® EHA105 (pSoup) 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:

- EHA105 Chem. Competent Agrobacterium (Cat.# 1084-06)
- LBA4404 Chem. Competent Agrobacterium (Cat.# 1085-06)
- GV3101 ElectroCompetent Agrobacterium (Cat.# 1282-12)
- 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.

Intact Genomics owns the following registered trademarks granted by the United States Patent and Trademark Office (USPTO): Intact Genomics®, IG®, ig®, igTherapeutics®, FastAmp®, i7®, DirectPlate®.

All technology protocols discussed within this manual are assumed proprietary to Intact Genomics. This Product may be covered by pending or issued patents or may have certain limitations. Please contact us for more information. Purchase of this material conveys to buyer the non-transferable right to use the material purchased in research conducted by buyer, whether for teaching, non-commercial or commercial research purposes. Buyer may not sell or otherwise transfer these materials, its components, or unmodified descendants to a third party.

Product Use Limitation and Disclaimers

This product is for research purposes only. It is not intended for therapeutic or diagnostic purposes in humans or animals. This product contains chemicals which may be harmful if misused or direct human contact is made.

Intact Genomics is dedicated to practicing and maintaining science and technology ethics. Buyer agrees to use the purchased materials in full compliance with applicable law and regulations.

Technical Support & Customer Services

Intact Genomics (IG®) is dedicated to customer satisfaction regarding the use of our products for your research needs. Each new lot of our products is thoroughly tested to ensure it meets high quality standards and provides excellent results. We appreciate your business and your feedback regarding the performance of our products in your applications. Please follow the instructions carefully and contact us if additional assistance is needed.

Our hours are Monday - Friday, 8AM to 5PM, U.S. Central Standard Time.

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
Website: www.intactgenomics.com