AGL1 ElectroCompetent Agrobacterium

Manual

Catalog #	1283-12	1283-20	1283-36
Package Size	6x50 µl	10x50 µl	18x50µl

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®) Agrobacterium *tumefaciens* AGL1 (AGL-1) cells are optimized for the highest transformation efficiencies which is ideal for applications requiring high transformation efficiencies, such as with cDNA or gDNA library construction. The AGL1 strain has a C58 chromosomal background that carries an insertion mutation in its recA recombination gene which stabilizes recombinant plasmids. It also carries rifampicin and carbenicillin resistance in its genome for selection. AGL1 contains the Ti plasmid pTiBO542 from which the T-DNA region sequences have been deleted. Transformation with a binary vector containing the missing T-region results in a functional T-DNA binary system that allows for transfer of genetic material into a host plant's genome. Therefore, this system is often used for Agrobacterium-mediated transformation of Arabidopsis thaliana as well as maize and other monocots.

Specifications:

Competent cell type: ElectroCompetent

Species: *A. tumefaciens*

Strain: AGL1

Format: Tubes

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

Blue/white screening: No

Shipping condition: Dry ice

Product Components:

- ig® AGL1 Electrocompetent Agrobacterium
- DNA (pCAMBIA1391z, 500 pg/µl)
- Recovery medium

Storage:

- ig® AGL1 ElectroComp. 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 ≥1 x 10^7 CFU/µg pCAMBIA1391z DNA. Untransformed cells are tested for appropriate antibiotic sensitivity.

General Guidelines:

Follow these guidelines when using AGL1 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 50 colonies, the TE is calculated as follows:

Colonies = 50

µg of DNA = 0.0005

Dilution = 100/1000 = 0.1

TE = 50/.0005/.1 = $1x10^7$

Transformation Protocol:

Use this procedure to transform ig® AGL1 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:

- AGL1 Chem. Competent Agrobacterium (Cat.# 1083-12)
- LBA4404 Chem. Competent Agrobacterium (Cat.# 1085-12)
- 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.

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