AGL1 Chemically Competent Agrobacterium

Intact Genomics®

Catalog #	1082-06	1082-18
Package Size	6x50 µl	18x50 µl

Description

Intact Genomics (ig®) Agrobacterium tumefaciens AGL1 (AGL -1) 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 carbenicilin 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:	Chemically competent
Species:	*A. tumefaciens*
Strain:	AGL1
Format:	Tubes
Transformation efficiency:	≥ 1 x 10^5 cfu/µg pCAMBIA1391z DNA
Blue/white screening:	No
Shipping condition:	Dry ice

Reagents Included

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

Note: Liquid nitrogen is required.

Storage

- AGL1 Chemically Comp. Agrobacterium: -80 ºC
- 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 10^5 CFU/µg pCAMBIA1391z DNA. Untransformed cells are tested for appropriate antibiotic sensitivity.

General Guidelines

Follow these guidelines when using AGL1 Chemically Competent Agrobacterium 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) pCAMBIA1391z control plasmid into 50 µl of cells, add 950 µl of Recovery Medium. Recover for 3 hours and plate 100 µl. Count the colonies on the plate in two days. If you count 5 colonies, the TE is calculated as follows:

Colonies = 5
µg of DNA = 0.0005
Dilution = 100/1000 = 0.1
TE = 5/.0005/.1 = 1x10^5

Transformation Protocol

Use this procedure to transform ig® AGL1 Chemically Competent Agrobacterium cells . Do not use these cells for electro competent transformation.

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 (10pg -1 µg) of DNA to the chilled micro-centrifuge 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 pCAMBIA1391z 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 for 5 minutes.
6) Incubate tubes for additional 5 minutes in 37°C water bath.
7) Immediately add 950µl of Recovery Medium or any other medium of choice to the tube, pipette up and down three times to re-suspend the cells.
8) Incubate tubes at 30 °C for 3 hours at 200 RPM.
9) 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.
10) Incubate the plates for 2 - 3 days at 30 °C.

Related Products

- GV3101 Chem. Competent Agrobacterium (Cat.# 1082-12)
- LBA4404 Chem. Competent Agrobacterium (Cat.# 1085-12)
- AGL1 ElectroCompetent Agrobacterium (Cat.# 1283-12)
- Agrobacterium Chemical Combo Pack (Cat.# 1090-24)
- T4 DNA Ligase (Cat.# 3212)

Technical Support

Intact Genomics is committed to supporting the worldwide scientific research community by supplying the highest quality reagents. Each new lot of our products is tested to ensure they meet the quality standards and specifications designated for the product.

Please follow the instructions carefully and contact us if additional assistance is needed. We appreciate your business and your feedback regarding the performance of our products in your applications.