High Efficiency AGL1 ElectroComp Agrobacterium

Description

Intact Genomics 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 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:	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

Reagents Needed for One Reaction

AGL1 ElectroComp Agrobacterium:	25 µl
DNA (pCAMBIA1391z, 100 pg/µl):	1 µl
Recovery medium:	1 ml

Storage

AGL1 ElectroComp 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 10^7 CFU/µg pCAMBIA1391z DNA. Untransformed cells are tested for appropriate antibiotic sensitivity.

General Guidelines

Follow these guidelines when using AGL1 ElectroComp 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.

Note: A high-voltage electroporation apparatus such as Bio-Rad Gene Pulser II #165-2105, capable of generating field strengths of 16 kV/cm is required.

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 (100 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 AGL1 ElectroComp Agrobacterium. 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 pCAMBIA1391z control, add 1 µl of (100 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 976 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 50 µl of diluted transformants 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.

Electroportation settings

Mode	Exponential protocol
Voltage (V)	1,800 V
Capacitance	25 uFD
Resistance	200 Ohms
Cuvette	1 mm