C58C1 Chemically Competent Agrobacterium

Manual

Catalog #	1086-06	1086-10	1086-18
Package Size	6x50µl	10x50µl	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®) C58C1 Chemically Competent Agrobacterium cells are optimized for the highest transformation efficiency and are useful for various applications. The chromosomal background of C58C1 is C58. C58 is cured of the Ti plasmid pTiC58 resulting in C58C1. C58C1 Competent cells may be useful for transgenic operations that involve Arabidopsis and other plants. This Agrobacterium strain is streptomycin and rifampicin-resistant.

Specifications:

Competent cell type: Chemically Competent

Species: *A. tumefaciens*

Strain: C58C1

Format: Tubes

Transformation efficiency: ≥ 1 x 10^5 cfu/µg pCAMBIA1391z DNA

Blue/white screening: No

Shipping condition: Dry ice

Reagents Included:

- ig® C58C1 Chemically Competent Agrobacterium
- DNA (pCAMBIA1391z, 500 pg/µl)
- Recovery medium

Note: Liquid nitrogen is required. All agrobacterial strains are not well studied for antibiotic resistance and there are many agrobacterial strains. Therefore, it is the customer's responsibility to make sure his/her vectors are compatible with the Agrobacterial strains if he/she uses an alternate antibiotic selection than kanamycin-selection.

Storage:

- C58C1 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 C58C1 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 = 1×10^5

Transformation Protocol:

Use this procedure to transform C58C1 Chemically Competent Agrobacterium cells. Do not use these cells for electrocompetent 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 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 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 a sterilized spreader or autoclaved ColiRoller™ plating beads to spread evenly.
10) Incubate the plates for 2 – 3 days at 30 °C.

Related Products:

- AGL1 Chem. Competent Agrobacterium (Cat.# 1083-12)
- LBA4404 Chem. Comp. Agrobacterium (Cat.# 1085-12)
- GV3101 ElectroComp.Agrobacterium (Cat.# 1282-12)
- Agrobacterium Chem. Combo Pack (Cat.# 1090-24)
- 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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