Ar.A4 Electrocompetent Agrobacterium

Manual

Catalog #	1272-12	1272-36
Package Size	6x50 µl	18x50 µl

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®) Ar.A4 Electrocompetent Agrobacterium cells are made from a specific strain of Rhizobium *rhizogenes (*formerly Agrobacterium *rhizogenes)*, Agrobacterium *rhizogenes* (kan R) Ar A4 Ri (agropine type). Agrobacterium *rhizogenes* is a soil-borne gram-negative bacterium that can infect most dicotyledons, a few monocotyledons, and some gymnosperms. Ar.A4 Electrocompetent Agrobacterium are optimized for the highest transformation efficiencies and are useful for transgenic operations of corn, tobacco, carrot, and other plants. Ar.A4 Agrobacterium *rhizogenes* strain contains agrobacterium-type Ri plasmid and displays kanamycin resistance.

Specifications:

Competent cell type: Electrocompetent

Species: *R. rhizogenes*

Strain: Ar.A4

Format: Tubes

Transformation efficiency: ≥ 1 x 10^7 cfu/µg pIG7-spe DNA

Blue/white screening: No

Shipping condition: Dry ice

Product Components:

- ig® Ar.A4 Electrocompetent Agrobacterium
- DNA (pIG7-spe, 500 pg/µl)
- Recovery medium

Note: 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:

- ig® Ar.A4 Electrocompetent Agrobacterium: -80 ºC
- pIG7-spe control DNA: -20 ºC
- Recovery medium: 4 ºC

Quality Control:

Transformation efficiency is tested by using the pIG7-spe control DNA supplied with the kit and using the protocol in this manual. Transformation efficiency should be ≥1 x 10^7 CFU/µg pIG7-spe DNA. Untransformed cells are tested for appropriate antibiotic sensitivity.

General Guidelines:

Follow these guidelines when using Ar.A4 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.

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 (500 pg/µl) pIG7-spe 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 = 50

µg of DNA = 0.0005

Dilution = 100/1000 = 0.1

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

Transformation Protocol:

Use this procedure to transform Ar.A4 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 pIG7-specontrol, 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 pIG7-spe control, you may plate 100 µl of undiluted transformation mix onto a YT plate containing 100 µg/ml spectinomycin. 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:

- GV3101 Chem. Competent Agrobacterium (Cat.# 1082-12)
- LBA4404 Chem. Competent Agrobacterium (Cat.# 1085-12)
- EHA105 ElectroCompetent Agrobacterium (Cat.# 1284-12)
- 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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Our hours are Monday - Friday, 8AM to 5PM, U.S. Central Standard Time.

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