

## Ar.A4 Chemically Competent Agrobacterium



<b>Catalog #</b>	1072-06	1072-18
<b>Package Size</b>	6x50 µl	18x50 µl

### Description

Intact Genomics (ig®) Ar.A4 Chemically Competent Agrobacterium cells are made from a specific strain of 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 Chemically Competent 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:	Chemically competent
Species:	<i>A. rhizogenes</i>
Strain:	Ar.A4
Format:	Tubes
Transformation efficiency:	≥ 1 x 10 <sup>5</sup> cfu/µg pIG7spe DNA
Blue/white screening:	No
Shipping condition:	Dry ice

### Reagents Included

- ig® Ar.A4 Chemically Competent Agrobacterium
- DNA (pIG7spe, 500 pg/µl)
- Recovery medium

**Note:** Liquid nitrogen is required.

### Storage

- Ar.A4 Chemically Comp. Agrobacterium: -80 °C
- pIG7spe control DNA: -20 °C
- Recovery medium: 4 °C

### Quality Control

Transformation efficiency is tested by using the pIG7spe control DNA supplied with the kit and using the protocol in this manual. Transformation efficiency should be ≥ 1 x 10<sup>5</sup> CFU/µg pIG7spe DNA. Untransformed cells are tested for appropriate antibiotic sensitivity.

### General Guidelines

Follow these guidelines when using Ar.A4 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 1 µg of plasmid into a given volume of competent cells.

$$TE = \text{Colonies}/\mu\text{g}/\text{Plated}$$

Transform 1 µl of (500 pg/µl) pIG7spe 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:

$$\begin{aligned} \text{Colonies} &= 5 \\ \mu\text{g of DNA} &= 0.0005 \\ \text{Dilution} &= 100/1000 = 0.1 \\ \text{TE} &= 5/.0005/.1 = 1 \times 10^5 \end{aligned}$$

### Transformation Protocol

Use this procedure to transform ig® Ar.A4 Chemically Competent Agrobacterium cells. Do not use these cells for electro competent transformation.

- Place microcentrifuge tubes on ice.
- Remove competent cells from the -80 °C freezer and thaw completely on wet ice (10-15 minutes).
- Aliquot 1 µl (10pg -1 µg) of DNA to the chilled microcentrifuge tubes on ice.
- 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 pIG7spe 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.
- Keep tubes on ice for 5 minutes, and then transfer to liquid nitrogen for 5 minutes.
- Incubate tubes for additional 5 minutes in 37°C water bath.
- 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.
- Incubate tubes at 30 °C for 3 hours at 200 RPM.
- Dilute the cells as appropriate then spread 20-200 µl cells onto a pre-warmed selective plate. For the pIG7spe 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.
- Incubate the plates for 2 - 3 days at 30 °C.

### Related Products

- GV3101 Chem. Competent Agrobacterium (Cat.# 1082-12)
- EHA105 Chem. Competent Agrobacterium (Cat.# 1084-12)
- Ar.A4 ElectroCompetent Agrobacterium (Cat.# 1272-12)

## AR.A4 Chemically Competent Agrobacterium



- 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.

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