# Protocol



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# EHA105 (pSuperAgro® v4tet) Electrocompetent Cells

## Transformation Protocol

## Introduction

GoldBio's EHA105 (pSuperAgro® v4tet) Electrocompetent Agrobacterium cells combine the widely used hypervirulent EHA105 strain with the enhanced pSuperAgro® v4tet helper plasmid to improve Agrobacterium-mediated T-DNA transfer. Optimized for electroporation, these transformation-ready cells support stable and transient plant transformation, CRISPR/Cas genome editing, and binary vector introduction.

Each kit includes high-efficiency electrocompetent cells, Agrobacterium Recovery Medium, and pCAMBIA1391z control DNA for validating transformation performance.

## Materials

- EHA105 (pSuperAgro® v4tet) Agrobacterium Electrocompetent Cells (GoldBio Catalog # CC-634)
    - **EHA105 Strain was generated, and primary clone supplied by Dr. Elizabeth Hood.**
- pCAMBIA1391z Control DNA, 10 ng/µl
- *Agrobacterium* Recovery Medium
- Kanamycin (GoldBio Catalog # K-120)
- Rifampicin (GoldBio Catalog # R-120)
- Tetracycline (GoldBio Catalog # T-101)
- Yeast Extract Tryptone (YT) or LB Agar selection plates.
- Microcentrifuge tubes
- Shaker incubator
- Sterile electroporation cuvettes
- Microcentrifuge tubes
- Electroporator

## Recommended Antibiotic Selection

Use the antibiotic required by the binary vector plus the strain/helper plasmid selection antibiotics below. Lower tetracycline is recommended because high tetracycline concentrations can strongly inhibit growth.

| Selection target | Antibiotic | Recommended concentration | Notes |
|---|---|---|---|
| EHA105 background | Rifampicin | 5 µg/mL | Strain selection/maintenance |
| pSuperAgro® v4tet helper plasmid | Tetracycline | 2 µg/mL | Helper plasmid maintenance |
| pCAMBIA1391z control plasmid | Kanamycin | 50 µg/mL | Control plasmid selection |
| User binary vector | Vector-dependent | Use vector requirement | Select as appropriate for vector |

- Suggested pCAMBIA1391z control plate: YT or LB agar containing rifampicin 5 µg/mL, tetracycline 2 µg/mL, and kanamycin 50 µg/mL.
- Suggested no-DNA controls: nonselective YT/LB to confirm recovery and YT/LB with rifampicin 5 µg/mL plus tetracycline 2 µg/mL to confirm strain/helper-plasmid growth.

**Note: Do not use the 50 µg/mL tetracycline disc-sensitivity value (as indicated on the chart below) as a routine growth or maintenance concentration for this strain.**

## Storage and Handling

- This product may be shipped on dry ice. EHA105 *Agrobacterium* Electrocompetent Cells should be stored at -80°C, pCAMBIA1391z Control DNA, 500 pg/µl, should be stored at -20°C and recovery medium should be stored at 4°C immediately upon arrival. When stored under the recommended conditions and handled correctly, these products should be stable for at least 1 year from the date of receipt.

- Thaw EHA105 *Agrobacterium* Electrocompetent Cells and pCAMBIA1391z Control DNA on ice and mix by gently tapping the tube. After thawing, these products should be kept on ice before use. These products can be refrozen for storage, but the transformation efficiency may decrease.

**Note: Transformation efficiency is tested by using the pCAMBIA1391z control DNA supplied with the kit and using the protocol given below. Untransformed cells are tested for appropriate antibiotic sensitivity.**

## Method

Transformation Protocol

Use this procedure to transform EHA105 (pSuperAgro® v4tet) *Agrobacterium* Electrocompetent cells. Do not use these cells for chemical transformation.

**Note: Handle the competent cells gently as they are highly sensitive to changes in temperature or mechanical lysis caused by pipetting.**

**Note: 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.**

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 (10 pg-1 ng) of DNA to the chilled microcentrifuge tubes on ice.

   **Note: DNA amounts above 1 ng will give decreasing transformation efficiencies.**

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 (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. For electroporation settings, check the manufacturer's handbooks.

6. Immediately add 975 µl of Recovery Media to the cuvette, gently pipette up and down three times to resuspend the cells. Transfer the cells and Recovery Medium to a culture tube.

7. Incubate at 30°C for 3 hours at 200 rpm in a shaker incubator.

8. Dilute the cells as appropriate, then spread 2 to 200 µl cells onto a pre-warmed selective plate. For the pCAMBIA1391z control, plate 50 µl of the diluted transformants onto a YT or LB plate containing 5 µg/ml rifampicin to select for the *Agrobacterium* as well as and 50 µg/ml kanamycin to select for the pCAMBIA control. Use a sterilized spreader or autoclaved plating beads to spread evenly.

   **Note: Dry plates for at least 25 minutes in a biohood for best results.**

   **Note: For best results, we recommend spreading 2 µl, 20 µl and 200 µl onto separate plates for each transformation. For the 2 or 20 µl plates, add 200 µl of recovery media to help spread. This helps to save time if transformation efficiencies are either very low or very high.**

9. Incubate the plates for 2-3 days at 30°C.

Table 1. Antibiotic Disc Sensitivity for GoldBio's *Agrobacterium* Strains (using standard BD antibiotic discs) * For reference only, not for culture selection use.

| Competent cells | Antibiotic Selection | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Amp 100 µg/ml | Carb 100 µg/ml | Chlor 30 µg/ml | Chlor 100 µg/ml | Gent 30 µg/ml | Kan 50 µg/ml | Rif 25 µg/ml | Spec 50 µg/ml | Strep 50 µg/ml | Tet 50 µg/ml |
| GV3101 | I | R | R | PR | R | S | R | S | R | S |
| EHA 105 | R | R/S | R | N/A | R/S | S | R | S | R | S |
| LBA 4404 | S | S | S | N/A | S | S | R | S | R | S |
| AGL-1 | R | R | R | N/A | R/S | S | R | S | R | S |
| C58C1 | R | R | R | N/A | R/S | S | R | S | R | S |

S = Sensitive
R = Resistant
R/S= intermediate zones using standard discs.
I= growth in inhibitory zone with standard disc. "Opaque", not clear zone of inhibition.

## 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

Where:
Colonies = the number of colonies counted
µg = amount of DNA transformed in µg
Dilution = total dilution of the DNA before plating

***Example:***
*Transform 1 µl of (500 pg/µl) pCAMBIA1391z control plasmid into 25 µl of cells, add 975 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 in 10 pg = 0.0005*
*Dilution = 100/1000 = 0.1*

*TE = 500/0.0005/0.1 = 1 x $10^7$*

## Associated Products

- GV3101 (pSuperAgro® v4tet) (GoldBio Catalog # CC-614)
- AGL-1 (pSuperAgro® v4) (GoldBio Catalog # CC-624)
- LBA4404 (pSuperAgro® v4) (GoldBio Catalog # CC-644)
- C58C1 (pSuperAgro® v4) (GoldBio Catalog # CC-654)
- Competent Cell Recovery Media (GoldBio Catalog # CC-300)
- Rifampicin (GoldBio Catalog # R-120)
- Kanamycin (GoldBio Catalog # K-120)