

Auxo-Agro™ EHA105 Chemically Competent Cells Transformation Protocol

Introduction

GoldBio's new Auxo-Agro™ competent cells are methionine auxotrophic strains of *Agrobacterium* which eliminates the need for antibiotics during transformation while increasing plant transformation efficiency.

Our EHA105 strain of *Agrobacterium tumefaciens* can be used in genetic transformation of tomato, tobacco and other plants. After transformation, antibiotics are commonly used to remove *Agrobacterium*. However, antibiotics have been shown to adversely affect plant germination and tissue growth. Goldbio Auxo-Agro™ EHA105 is an EHA105ΔMet1 *Agrobacterium tumefaciens* cell line that is methionine dependent, which both minimizes the use of antibiotics and simplifies the transformation procedure. Goldbio Auxo-Agro™ auxotrophic strains eliminate the need for antibiotics. Instead, the removal of methionine from the culture media for the Auxo-Agro™ line of *Agrobacterium* automatically induces *Agrobacterium* cell death.

GoldBio's EHA105 *Agrobacterium* chemically competent cells allow you to obtain high transformation efficiency in applications such as gDNA or cDNA library construction. Our EHA105 strain contains a rifampicin resistance gene (rif) and an amber basic Ti plasmid pEHA105 (pTiBo542DT-DNA) without self-transport function, containing the vir gene.

Materials

- Auxo-Agro™ EHA105 Chemically Competent Cells (GoldBio Catalog # CC-168)
 - **Strain was generated, and primary clone supplied by Dr. Wayne Parrott under license from his institution.**
- pCAMBIA1391z Control DNA, 10 ng/μl
- *Agrobacterium* Recovery Medium
- Kanamycin (GoldBio Catalog # K-120)
- Rifampicin (GoldBio Catalog # R-120)
- Yeast Extract Tryptone (YT) or LB Agar selection plates.
- Microcentrifuge tubes
- Shaker incubator
- Liquid nitrogen

Storage and Handling

- This product may be shipped on dry ice. Auxo-Agro™ EHA105 Chemically Competent Cells should be stored at -80°C, pCAMBIA1391z Control DNA, 10 ng/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 Auxo-Agro™ EHA105 Chemically Competent 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.

Note: Transformation efficiency is tested by using the pCAMBIA1391z control DNA supplied with the kit and using the protocol given below. Transformation efficiency should be $\geq 1 \times 10^4$ cfu/μg pCAMBIA1391z DNA. Untransformed cells are tested for appropriate antibiotic sensitivity.

Method

Transformation protocol

Use this procedure to transform Auxo-Agro™ EHA105 Chemically Competent Cells. Do not use these cells for electroporation.

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 microcentrifuge tubes on ice.
2. Remove competent cells from the -80°C freezer and thaw completely on ice (10-15 minutes).
3. Aliquot 1 μl (50 ng to 500 ng) of DNA to the chilled microcentrifuge tubes on ice.

Note: It is important to use more DNA than in a typical bacterial transformation. We recommend using ~500 ng, if possible.

4. When the cells are thawed, add 50 μl of cells to each DNA tube on ice and mix gently by tapping 4-5 times. For the pCAMBIA1391z control, add 5 μl of (10 ng/μl) DNA to 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 a dry ice/ethanol bath or liquid nitrogen for 5 minutes.

Note that if you used a dry ice/ethanol bath you can place the frozen tubes on dry ice for up to 30 minutes prior to the 37°C heat shock.

Note: For a dry ice/ethanol bath, we recommend adding ~200 ml EtOH to a used pipet tip container and slowing adding dry ice over the course of 10 minutes.

6. Incubate tubes in a 37°C water bath for 2 minutes (for dry ice/ethanol bath) or for 5 minutes (for liquid nitrogen).
7. Immediately add 950 µl of Recovery Media and gently pipette up and down three times to resuspend the cells.
8. Incubate at 30°C for 3 hours at 200 rpm in a shaking incubator.
9. Dilute the cells as appropriate, then spread 2 to 200 µl cells onto a selective plate. For the pCAMBIA1391z control, plate 200 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.

10. 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)

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.

Calculations

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.

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

Where:

Colonies = the number of colonies counted

µg = amount of DNA transformed in µg

Dilution = total dilution of the DNA before plating

Example:

Transform 5 µl of (10 ng/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 the next day. If you count 500 colonies, the TE is calculated as follows:

Colonies = 500

µg of DNA = 0.05

Dilution = 100 µl/1000 = 0.1

$TE = 500/0.05/0.1 = 1.0 \times 10^5$

Related Products

- Auxo-Agro™ EHA105 Electrocompetent Cells (GoldBio Catalog # CC-268)
- Auxo-Agro™ LBA4404 Chemically Competent Cells (GoldBio Catalog # CC-167)
- Auxo-Agro™ LBA4404 Electrocompetent Cells (GoldBio Catalog # CC-267)
- EHA 105 *Agrobacterium* Chemically Competent Cells (GoldBio Catalog # CC-108)
- LBA4404 *Agrobacterium* Chemically Competent Cells (GoldBio Catalog # CC-107)
- AGL-1 *Agrobacterium* Chemically Competent Cells (GoldBio Catalog # CC-106)
- GV3101 *Agrobacterium* Chemically Competent Cells (GoldBio Catalog # CC-105)
- C58C1 *Agrobacterium* Chemically Competent Cells (GoldBio Catalog # CC-109)
- Competent Cell Recovery Media (GoldBio Catalog # CC-300)
- Rifampicin (GoldBio Catalog # R-120)
- Kanamycin (GoldBio Catalog # K-120)