GeneArt® MAX Efficiency® Transformation Reagent for Algae

**Description**

Introduction of exogenous DNA into the unicellular, green alga *Chlamydomonas reinhardtii* is hindered by the organism’s rigid cell wall. Although various methods, such as glass beads agitation, electroporation, and microparticle bombardment, have been successfully used to transform *C. reinhardtii*, they provide very low transformation efficiency. The GeneArt® MAX Efficiency® Transformation Reagent facilitates the delivery of DNA into the cell during electroporation, providing 2 to 3 orders of magnitude increase in transformation efficiency compared to conventional electroporation methods.

<table>
<thead>
<tr>
<th>Product</th>
<th>Catalog No.</th>
<th>Amount</th>
<th>Storage</th>
<th>Shelf Life*</th>
</tr>
</thead>
<tbody>
<tr>
<td>GeneArt® MAX Efficiency® Transformation Reagent</td>
<td>A24229</td>
<td>250 mL</td>
<td>Store at 2°C to 8°C.</td>
<td>6 months</td>
</tr>
</tbody>
</table>

*Shelf Life duration is determined from Date of Manufacture.

**Product Use**

For Research Use Only. Not for use in diagnostic procedures.

**Safety Information**

Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

**Culture Conditions for *C. reinhardtii***

**Media:** Gibco® TAP medium (Cat. no. A13798)

**Culture Type:** Routine maintenance is usually done at room temperature on 1.5% agar, while growth for individual experiments is typically done in liquid culture in shake flasks or bottles.

**Temperature Range:** Optimum temperature for the growth of *C. reinhardtii* 137c is 26°C, but *C. reinhardtii* laboratory and wild type strains grow well in the range of 20–28°C and can tolerate temperatures as low as 15°C and as high as 35°C.

**Incubation Conditions:** Phototrophic cultures should be supplied with 5% CO₂ for maximal growth and incubated under continuous illumination using moderate light intensities of cool fluorescent white light (50 ± 10 µE m⁻² s⁻¹) with constant agitation on a gyrotary shaker set to 100–150 rpm. However, the *C. reinhardtii* 137c strain can grow in the incubator without the need of additional CO₂ supply. Ensure that proper gas exchange is achieved in culture vessels. After transformation and plating, do not stack the culture plates to allow continuous uniform illumination.

**Recommended Equipment:** The optimal equipment for culturing *C. reinhardtii* is an algal growth chamber (e.g., Percival Algal Chamber from Geneva Scientific) with regulatable light supply and a temperature on 1.5% agar, while growth for individual experiments can be achieved with conventional electroporation methods. However, the *C. reinhardtii* laboratory and wild type strains grow well in the range of 20–28°C and can tolerate temperatures as low as 15°C and as high as 35°C.

**Guidelines for transforming *C. reinhardtii***

- Nuclear transformation of *C. reinhardtii* can be achieved with circular DNA; however, transformation with linearized DNA is much more efficient. To get the maximum number of colonies, we recommend linearizing your vector. If you are transforming with pChlamy vectors from Life Technologies, we recommend using *SacI* restriction enzyme for linearization, provided that the insert does not contain the recognition sequence for *SacI*. Otherwise, you can choose from *PvuI*, *SpI*, or *FspI*.
- We recommend using 2 µg of linearized plasmid DNA per electroporation.
- The quality and the concentration of DNA used play a central role for the efficiency of transformation. Use a commercial kit such as the PureLink® HQ Mini Plasmid Purification Kit (Cat. no. K2100-01) or the PureLink® HiPure Plasmid Miniprep Kit (Cat. no. K2100-02) that delivers pure DNA.
- For best results, grow the cells to 1 × 10⁶–2 × 10⁶ cells/mL before proceeding with electroporation. You may use <1 × 10⁶ cells/mL, but the concentration should not exceed 3 × 10⁶ cells/mL.
- Insertion of the plasmid DNA into the genome occurs randomly. As an optional step, you may first screen the colonies by colony PCR to ensure full integration of the promoter and the gene of interest, followed by the screening of several positive clones for the expression of the gene of interest to pick the highest expressing clone.
- Because the *C. reinhardtii* genome has a very high GC content (~62% GC), the expression levels of recombinant genes are significantly improved if the gene of interest is adapted to the preferred codon usage of highly expressed *C. reinhardtii* genes.

**Materials Needed**

- pChlamy vector construct containing your gene of interest and linearized with the appropriate restriction enzyme
- Gibco® TAP medium (Cat. no. A13798), pre-warmed to room temperature
- TAP-40 mM sucrose solution, pre-warmed to room temperature
- Selective TAP-Agar plates: TAP-Agar-Hygromycin plates (10 µg/mL) or TAP-Agar-ZeoCl™ plates (5 µg/mL)
- Sterile 15-mL and 50-mL centrifugation tubes
- 0.4-cm electroporation cuvettes (Cat. no. P460-50), chilled on ice
- Electroporation device such as the Bio-Rad® Gene Pulser® II

**Optional:** Alternatively, you can use the Neon® Transfection System (Cat. no. MPK5000) or the Neon® Transfection System 100 µL Kit (Cat. no. MPK10025).

**Prepare Media and Reagents**

**TAP-40 mM sucrose solution**

1. Prepare 1 M sucrose stock solution by mixing 342.3 g of sucrose to 1 L of deionized water and adding water to bring the final volume to 1 L. Filter sterilize the 1 M sucrose solution through a 0.22 µm filter.
2. To prepare the TAP-40 mM sucrose solution, add 44 mL of 1 M sucrose to 1 L of Gibco® TAP medium.

**Selective TAP-Agar plates**

1. Add 15 g of agar to 200 mL of Gibco® TAP medium and autoclave on liquid cycle for 20 minutes.
2. Warm 800 mL of Gibco® TAP medium to 55–60°C in a water bath.
3. After autoclaving, cool the agar containing flask to ~55°C.
4. Combine the agar containing flask with 800 mL of Gibco® TAP medium, add hygromycin B to a final concentration of 10 µg/mL or Zeocin® to a final concentration of 5 µg/mL, and pour into 10 cm plates.
5. Let the plates harden (do not overdry), inversion them, and store at 4°C in the dark. Final agar concentration will be 1.5%.

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Transform *C. reinhardtii* using BioRad® Gene Pulser® II

1. Revive 1 vial of frozen *C. reinhardtii* cells and inoculate into 200 mL of TAP medium. Culture the cells under standard conditions and keep monitoring their concentration for 3 days.
2. When cell concentration reaches \(1 \times 10^6 - 2 \times 10^6\) cells/mL, harvest them by centrifugation at 2500 rpm for 5 minutes. Discard the supernatant and carefully remove all liquid as much as possible.

   **Note:** Cells must be in early log phase and harvested gently. If the cell concentration is <1 \(\times 10^6\) cells/mL, you may still harvest the cells without significantly affecting the transformation efficiency.
3. Resuspend the cell pellet in 10 mL of GeneArt® MAX Efficiency® Transformation Reagent and centrifuge at 2500 rpm for 5 minutes. Discard the supernatant, and carefully remove all liquid as much as possible.
4. Resuspend the pellet again in 10 mL of GeneArt® MAX Efficiency® Transformation Reagent and centrifuge once more at 2500 rpm for 5 minutes.
5. Resuspend the cells in GeneArt® MAX Efficiency® Transformation Reagent to a final concentration of \(2 \times 10^6 - 3 \times 10^6\) cells/mL.
6. Add 2–4 µg of linearized DNA per 250 µL of cell suspension and incubate at 4°C for 5 minutes.
7. Set electroporation parameters on the Gene Pulser® II as follows:

<table>
<thead>
<tr>
<th>Voltage</th>
<th>Capacity</th>
<th>Resistance</th>
</tr>
</thead>
<tbody>
<tr>
<td>500 V</td>
<td>50 µF</td>
<td>800 Ω</td>
</tr>
</tbody>
</table>

8. Transfer 250 µL of the cell-DNA mix into an ice-cold cuvette just before electroporation.
9. Electroporate the cells using the appropriate settings. Usually, the electro pulse duration is about 30 ms.
10. After electroporation, allow the cells to recover on the bench for 15 minutes.
11. Transfer the cells into a 50-mL conical tube or flask containing 10 mL of TAP-40 mM sucrose solution at room temperature.
12. Place the cells in the algal chamber and incubate for 14–16 hours.
13. Harvest the cells centrifugation at 2500 rpm for 5 minutes, discard the supernatant, and resuspend the pellet in 200 µL TAP medium.
14. Plate the cells on selective TAP-agar plate, and incubate in the algal chamber for 5–7 days.

   **Note:** The colony yield depends on the size of the plasmid DNA, the selection marker, and other factors.

Transform *C. reinhardtii* using Neon® Transfection System

For detailed instructions on using the Neon® Transfection System, refer to the Neon® Transfection System user guide, available for downloading at www.lifetechnologies.com.

1. Harvest the cells as described in Steps 1–3 of the GenePulser® II protocol, and resuspend them in GeneArt® MAX Efficiency® Transformation Reagent to a final concentration of \(1 \times 10^6 - 3 \times 10^6\) cells/mL.
2. Add 1 µg of linearized DNA per 100 µL of cell suspension and incubate at 4°C for 5 minutes.
3. Fill the Neon® Tube with 3 mL of ice-cold E2 buffer and insert it into the Neon® Pipette Station until you hear a click.

   **Note:** After 2–3 shocks, E2 buffer needs to be chilled on ice again.

4. Set electroporation parameters on the Neon® device as follows:

<table>
<thead>
<tr>
<th>Voltage</th>
<th>Pulse width</th>
<th>Pulse number</th>
</tr>
</thead>
<tbody>
<tr>
<td>2300 V</td>
<td>13 ms</td>
<td>3</td>
</tr>
</tbody>
</table>

5. Pipette up 100 µL of the DNA-cell mix in the 100-µL Neon® Tip and insert the tip into the Neon® Tube in the pipette station until you hear a click.
6. Press the Start button on the touchscreen to deliver the electric pulse.
7. Eject the electroporated cells into a 15-mL centrifuge tube (chilled on ice) and allow the cells to recover on the bench for 15 minutes.
8. Add 4 mL of TAP-40 mM sucrose solution at room temperature to the cells and incubate them in the algal chamber overnight.
9. The next day, centrifuge the cells at 2500 rpm for 5 minutes, discard 3.8 mL of the supernatant, and resuspend the cells in the remaining 200 µL of TAP-40 mM sucrose solution.
10. Spread 200 µL of the cell suspension on selective TAP-agar plates, and incubate in the algal chamber for 5–7 days.

**Related Products**

**Product** | **Cat. No.**
---|---
GeneArt® *Chlamydomonas* Protein Expression Kit | A24244
GeneArt® *Chlamydomonas* Engineering Kit | A14258
GeneArt® *Chlamydomonas* Engineering Kit with 6L media | A14262
GeneArt® *Chlamydomonas* TOPO® Engineering Kit | A14260
GeneArt® *Chlamydomonas* TOPO® Engineering Kit with 6L media | A14264
Gibco® TAP medium | A13798
Electroporation cuvettes, 0.4 cm | P460-50
PureLink® HQ Mini Plasmid Purification Kit | K2100-01
PureLink® HiPure Plasmid Miniprep Kit | K2100-02
Neon® Transfection System | MPK5000
Neon® Transfection System 100 µL Kit | MPK10025
Hygromycin B Selective Antibiotic | 10687-010
Zeocin™ Selection Reagent | R250-01

**Explanation of Symbols and Warnings**

The symbols present on the product label are explained below:

- **LOT**
- **REF**
- **Manufacturer**
- **Use By:**
- **Read SDS**
- **Read Safety Data Sheet**

**Limited Product Warranty**

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies’ General Terms and Conditions of Sale found on Life Technologies’ website at www.lifetechnologies.com/termsandconditions. If you have any questions, please contact Life Technologies at www.lifetechnologies.com/support.

**Important Licensing Information**

This product may be covered by one or more Limited Use Label Licenses. By use of this product, you accept the terms and conditions of all applicable Limited Use Label Licenses.

For additional technical information such as Safety Data Sheets (SDS), Certificates of Analysis, visit www.lifetechnologies.com/support.