GeneArt® Cryopreservation Kit for Algae

Description
The best method for the preservation and long-term storage of algae is cryopreservation, which dramatically reduces genetic drift, lowers labor and cost associated with the maintenance of algae large number of plates, and also facilitates strain and clone exchange between laboratories. In contrast to most cryopreservation methods that require liquid nitrogen storage, the GeneArt® Cryopreservation Kit for Algae allows algae to be frozen and stored in a –80°C freezer for at least 2 years. GeneArt® Cryopreservation Kit for Algae provides an optimized reagent and a robust cryopreservation protocol for Chlamydomonas reinhardtii and Chlorella vulgaris.

<table>
<thead>
<tr>
<th>Product</th>
<th>Catalog no.</th>
<th>Amount</th>
<th>Storage</th>
</tr>
</thead>
<tbody>
<tr>
<td>GeneArt® Cryopreservation Kit for Algae contains:</td>
<td>A24228</td>
<td>1 kit</td>
<td>2°C to 8°C</td>
</tr>
<tr>
<td>Cryopreservation Reagent A</td>
<td></td>
<td>5 mL</td>
<td></td>
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<tr>
<td>Cryopreservation Reagent B</td>
<td></td>
<td>25 mL</td>
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</table>

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°C to 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 gyratory 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 light meter (e.g., LI-250A Light Meter from LI-COR®) to guide adjustments. If an algal growth chamber is not available, the cells can be grown in a standard cell culture incubator illuminated with cool fluorescent lights placed within 12 inches of the culture plates.

Materials required but not provided
- Mr. Frosty® freezing container (Nalgene, Cat. no. 5100-0001)
- Benchtop centrifuge (e.g., Sorvall)
- Cryovials (Sarstedt, Cat. no. 72.685.701).
- Algal Growth Chamber (e.g., Percival® Algal Chamber from Geneva Scientific) set to 26°C, 50 µE m–2 s–1

Note: If an Algal Chamber is not available, you can use a standard cell culture incubator under continuous illumination using moderate intensities of cool fluorescent white light (50 µE m–2 s–1).
- Rotary shaking platform set to 110 rpm
- 250-mL clear-glass culture flask
- Gibco® TAP medium (Cat. no. A13798), pre-warmed to room temperature
- 70% ethanol
- Dry ice

Freezing Chlamydomonas reinhardtii cells
1. Grow C. reinhardtii cells (wild type or transformants) into mid- to late-logarithmic phase under standard culture conditions.
2. Prepare pre-conditioning medium in a 250-mL clear-glass culture flask by adding 1 mL of Cryopreservation Reagent A into 45 mL of fresh Gibco® TAP medium.
3. Inoculate the pre-conditioning medium with C. reinhardtii cells from step 1 to a final OD750 of 0.1 (usually, 2–5 mL of seed culture). Do not exceed OD750 of 0.4.
4. Place the culture flask on a rotary shaking platform set to 110 rpm in an algal growth chamber at 26°C and 50 µE m–2 s–1, and incubate for 3 days. You may let the cells grow in pre-conditioning medium for 2–5 days, but the optimal time is 3 days.
5. After 3 days of growth, measure the OD750 of the culture and calculate the cell concentration using the equation below.

\[
\text{Cell concentration (cells/mL) = (OD750 – 0.088)/(9 \times 10^{-5})}
\]

6. Optional: After 3 days growth under lighted conditions, the culture can be moved to dim light condition for overnight incubation before harvest (step 7, below). This optional step could increase cell viability during freezing.
7. Harvest the cells by centrifugation at 2500 rpm for 5 minutes and carefully remove as much of the supernatant as possible.
8. Resuspend the cells to a final concentration of 2.5 × 107 cells/mL in Cryopreservation Reagent B. Start counting the incubation time at this point (30–45 minutes at room temperature; see step 9, below).

Note: Do not exceed more than 5 × 107 cells/mL (cell viability will be dramatically reduced at higher concentrations).
9. Aliquot exactly 240 µL of cell suspension into each cryovial and incubate at room temperature for 30–45 minutes.
10. Remove the sponge insert from the Mr. Frosty® freezing container and directly insert the gray high-density polyethylene vial holder in its place. Transfer the cryovials containing the cells into the Mr. Frosty® freezing container. If you do not have 18 vials to occupy all of the slots of the vial holder, fill the rest of slots with similar liquid-filled cryovials to ensure a proper cooling profile. Do not fill the container with 100% isopropyl alcohol or any other freezing liquid.
11. Move Mr. Frosty® freezing container with the cryovials to –80°C. Place the Mr. Frosty® freezing container on an open space in the freezer to ensure that no other objects block the cooling process.
12. In the next 2 hours, make sure that the –80°C freezer remains unopened. Opening the freezer door during this period changes the cells’ cooling profile and may result in decreased cell viability.
13. After 4 hours, the cryovials can be transferred to another container for longer term storage at –80°C or remain in the Mr. Frosty® freezing container.
14. The cells can be stored at –80°C for at least 2 years. Note that this freezing protocol may also be suitable for other species of Chlamydomonas.

**Thawing Chlamydomonas reinhardtii cells**

1. Remove the frozen cells from –80°C storage and immediately place them in a dry ice container. Bury the vial(s) containing the cells in dry ice to minimize temperature fluctuations before thawing.
2. Add 200 mL of Gibco® TAP medium, pre-warmed to room temperature, into a 500-mL glass culture flask.
3. Remove the cryovial containing the frozen cells from the dry ice storage and immediately place it into a 35°C water bath.
4. Quickly thaw the cells by gently swirling the vial in the 35°C water bath until the cell have completely thawed (1–2 minutes).
5. Before opening, wipe the outside of the vial with 70% ethanol.
6. Transfer 230 µL of thawed cells from the vial into the glass culture flask containing 200 mL of Gibco® TAP medium.
7. Place the flask(s) in the algal growth chamber set to 26°C and 50 µE m⁻² s⁻¹.
8. Incubate the cells for 3–6 days with agitation on a rotary shaker set to 110 rpm.
9. On Day 3, count the cell number. If the culture has not yet reached 1 × 10⁸ cells/mL, return it to the algal growth chamber and continue the incubation. Check the cell concentration of the culture daily until it reaches 1 × 10⁸ cells/mL. Once the culture has reached 1 × 10⁸ cells/mL, proceed to the transformation step.

**Freezing Chlorella vulgaris cells**

1. Grow C. vulgaris cells (wild type or transformants) into mid- to late-logarithmic phase under standard culture conditions in 100 mL of Gibco® TAP medium.
2. Determine the cell concentration and the total cell number using the Countess® Automated Cell Counter or your preferred method.
3. Harvest the cells by centrifugation at 2500 rpm for 5 minutes and carefully remove as much of the supernatant as possible.
4. Resuspend the cells to a final concentration of 1 × 10⁶ cells/mL in Cryopreservation Reagent B. Start counting the incubation time at this point (30–45 minutes at room temperature; see step 5, below).
5. Note: Final cell concentration can range from 1 × 10⁴ to 2 × 10⁶ cells/mL, without affecting the viability of frozen cells.
6. Aliquot exactly 240 µL of cell suspension into each cryovial and directly insert the gray high-density polyethylene vial holder in its place. Transfer the cryovials containing the cells into the Mr. Frosty® freezing container. If you do not have 18 vials to occupy all of the slots of the vial holder, fill the rest of slots with similar liquid-filled cryovials to ensure a proper cooling profile. Do not fill the container with 100% isopropyl alcohol or any other freezing liquid.
7. Move Mr. Frosty® freezing container with the cryovials to –80°C. Place the Mr. Frosty® freezing container on an open space in the freezer to ensure that no other objects block the cooling process.
8. In the next 2 hours, make sure that the –80°C freezer remains unopened. Opening the freezer door during this period changes the cells’ cooling profile and may result in decreased cell viability.
9. After 4 hours, the cryovials can be transferred to another container for longer term storage at –80°C or remain in the Mr. Frosty® freezing container.
10. The cells can be stored at –80°C for at least 2 years. Note that this freezing protocol may also be suitable for other species of Chlorella.
11. Follow the thawing protocol provided for Chlamydomonas cells to resuscitate Chlorella frozen vials.

**Related products**

<table>
<thead>
<tr>
<th>Product</th>
<th>Cat. no.</th>
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<tr>
<td>GeneArt® Chlamydomonas Protein Expression Kit</td>
<td>A24244</td>
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<tr>
<td>GeneArt® Chlamydomonas Engineering Kit</td>
<td>A14258</td>
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<tr>
<td>GeneArt® Chlamydomonas Engineering Kit with 6L media</td>
<td>A14262</td>
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<tr>
<td>GeneArt® Chlamydomonas TOPO® Engineering Kit</td>
<td>A14260</td>
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<tr>
<td>GeneArt® Chlamydomonas TOPO® Engineering Kit with 6 L media</td>
<td>A14264</td>
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<tr>
<td>Gibco® TAP medium</td>
<td>A13798</td>
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<tr>
<td>Electroproporation cuvettes, 0.4 cm</td>
<td>P460-50</td>
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<tr>
<td>PureLink® HQ Mini Plasmid Purification Kit</td>
<td>K2100-01</td>
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<td>PureLink® HiPure Plasmid Miniprep Kit</td>
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<td>Neon® Transfection System</td>
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<td>Neon® Transfection System 100 µL Kit</td>
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<tr>
<td>Hygromycin B Selective Antibiotic</td>
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<td>Zeocin™ Selection Reagent</td>
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**Explanation of symbols and warnings**

The symbols present on the product label are explained below:

- **LOT**
- **REF**
- **STERILE A**
- **Read SDS**

**Limited product warranty**

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