invitrogen
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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*.

Product	Catalog no.	Amount	Storage
GeneArt® Cryopreservation Kit for Algae contains:	**A24228**	**1 kit**	2°C to 8°C
Cryopreservation Reagent A		5 mL	
Cryopreservation Reagent B		25 mL	

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% CO_2 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 CO_2 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. Standard room lights provide sub-optimal growth conditions.

Materials required but not provided

- Mr. Frosty® freezing container (Nalgene, Cat. no. 5100-0001)
- Benchtop centrifuge (e.g., Sorvall)
- Cryovials (Sarstaedt, 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 OD_{750} of 0.1 (usually, 2–5 mL of seed culture). Do not exceed OD_{750} 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 OD_{750} of the culture and calculate the cell concentration using the equation below.

   Cell concentration (cells/mL) = $(OD_{750} - 0.088)/(9 \times 10^{-8})$
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 2.5×10^7 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 5×10^7 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 1×10^6 cells/mL, return it to the algal growth chamber and continue the incubation. Check the cell concentration of the culture daily until it reaches 1×10^6 cells/mL. Once the culture has reached 1×10^6 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 1×10^8 cells/mL in Cryopreservation Reagent B. Start counting the incubation time at this point (30–45 minutes at room temperature; see step 5, below).

   Note: Final cell concentration can range from 1×10^7 to 2×10^8 cells/mL without affecting the viability of frozen cells.
5. Aliquot exactly 240 µL of cell suspension into each cryovial and incubate at room temperature for 30–45 minutes.
6. 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.
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

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 6 L 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			Read SDS
Batch Code	Catalog number	Manufacturer	Use By:	Read Safety Data Sheet
		STERILE A		
Caution, consult accompanying documents	Temperature Limitation	Sterilized using aseptic processing techniques		Consult instructions for use

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**.

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For further assistance, email **techsupport@lifetech.com**

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