



# *Euglena gracilis* Klebs

12894™

## Description

**Strain designation:** 1224-5/25 [CCAP 1224/5Z, Pringsheim Z, UTEX 753]

**Deposited As:** *Euglena gracilis* Klebs

**Type strain:** No

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## Storage Conditions

**Product format:** Frozen

**Storage conditions:** -80°C or colder for 1 week, vapor phase of liquid nitrogen for long-term storage

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## Intended Use

This product is intended for laboratory research use only. It is not intended for any animal or human therapeutic use, any human or animal consumption, or any diagnostic use.

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## BSL 1

ATCC determines the biosafety level of a material based on our risk assessment as guided by the current edition of *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, U.S. Department of Health and Human Services. It is your responsibility to understand the hazards associated with the material per your organization's policies and procedures as well as any other applicable regulations as enforced by your local or national agencies.

ATCC highly recommends that appropriate personal protective equipment is always

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used when handling vials. For cultures that require storage in liquid nitrogen, it is important to note that some vials may leak when submersed in liquid nitrogen and will slowly fill with liquid nitrogen. Upon thawing, the conversion of the liquid nitrogen back to its gas phase may result in the vial exploding or blowing off its cap with dangerous force creating flying debris. Unless necessary, ATCC recommends that these cultures be stored in the vapor phase of liquid nitrogen rather than submersed in liquid nitrogen.

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### Certificate of Analysis

For batch-specific test results, refer to the applicable certificate of analysis that can be found at [www.atcc.org](http://www.atcc.org).

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### Growth Conditions

**Medium:**

ATCC Medium 351: Hutner's medium for *Euglena*

**Instructions for complete medium:** ATCC Medium 351

**Temperature:** 25°C

**Culture system:** Axenic

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### Handling Procedures

#### Storage and Culture Initiation

Frozen ampules packed in dry ice should either be thawed immediately or stored in liquid nitrogen. If liquid nitrogen storage facilities are not available, frozen ampules may be stored at or below -70°C for approximately one week. **Do not under any circumstance store frozen ampules at refrigerator freezer temperatures (generally**

**-20°C).** Storage of frozen material at this temperature will result in the death of the culture.

1. To thaw a frozen ampule, place in a 35°C water bath, until thawed (2-3 min). Immerse the ampule just sufficient to cover the frozen material. Do not agitate the ampule.
2. Immediately after thawing, aseptically transfer contents to a 16 x 125 mm screw-capped borosilicate test tube containing ATCC Medium 351. Incubate the tube upright for one hour.
3. Gently remove as much supernatant as possible (**note: methanol can inhibit growth**) and refill with an equal volume of fresh medium.
4. Incubate on a horizontal slant at 50-100  $\mu\text{Einsteins}/\text{m}^2/\text{s}$  irradiance at 25°C with the cap loosened one half turn. Maintain under a 14/10 h light-dark photoperiod.

### **Culture maintenance:**

1. Inoculate a tube of fresh broth medium with 0.1 ml from a growing culture at or near peak density.
2. Incubate at 50-100  $\mu\text{Einsteins}/\text{m}^2/\text{s}$  irradiance at 25° C with the cap loosened one half turn. Maintain under a 14/10 h light-dark photoperiod.

### **Cryopreservation:**

1. Harvest cells from a culture which is at or near peak density by centrifuging at 100 x g for 1 minute  
**Note:** Centrifugation at the lowest speed and the shortest time to allow sedimentation of the cells will maximize recovery.
2. Adjust the concentration of cells to 4 x 10<sup>6</sup>/ml with fresh broth medium.
3. Transfer the concentrated cell suspension to a sterile Petri dish and allow the cells to remain undisturbed for at least one hour.
4. Transfer the cell suspension (note the volume) from the Petri plate to a 15 ml plastic centrifuge tube.
5. Add an equal volume of 6% (v/v) sterile reagent grade methanol solution that has been prepared in fresh ATCC medium 351.
6. Dispense in 0.5 ml aliquots into 1.0 - 2.0 ml sterile plastic screw-capped cryules (special plastic vials for cryopreservation). The time from mixing of the cell preparation and the methanol solution, before the cooling cycle begins, should be no greater than 15 min.
7. Place vials in a controlled rate freezing unit. From room temperature cool at -

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1°C/min to -40°C. If freezing unit can compensate for the heat of fusion, maintain rate at -1 C/min through heat of fusion. At -40°C plunge ampules into liquid nitrogen. Alternatively, place the vials in a Nalgene 1°C freezing apparatus. Place the apparatus at -80°C for 1.5 to 2 hours and then plunge ampules into liquid nitrogen. (The cooling rate in this apparatus is approximately -1°C/min.)

8. The frozen preparations should be stored in either the vapor or liquid phase of a nitrogen refrigerator. Frozen preparations stored below -130°C are stable indefinitely. Those stored at temperatures above -130°C are progressively less stable as the storage temperature is elevated. Vials can be stored between -80 and -70°C for no longer than one week.
9. To establish a culture from the frozen state follow steps 1-4 listed above under the heading **ESTABLISHING A CULTURE FROM A FROZEN AMPULE.**

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### Material Citation

If use of this material results in a scientific publication, please cite the material in the following manner: *Euglena gracilis* Klebs (ATCC 12894)

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### References

References and other information relating to this material are available at [www.atcc.org](http://www.atcc.org).

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