



# ***Plasmodium relictum*** **Grassi and Feletti**

**30141™**

## **Description**

**Strain designation:** 1P and 1P1

**Deposited As:** *Plasmodium relictum* Grassi and Feletti

**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.

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ATCC highly recommends that appropriate personal protective equipment is always 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

**Host:** In vivo cultivation in pigeons (age 3-4 d.)

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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 ampoules 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. The following directions for recovery from the frozen state must be carefully followed if a culture is to be successfully established.

1. Place the frozen vial in a 37°C water bath until mixture is completely thawed.

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2. Aseptically transfer the contents to a sterile syringe and inject into wing vein of host organism.
3. Make a smear if required (see below).

**Culture maintenance:** To monitor the infection (recommended every 24 hrs. from day 3 onwards), withdraw a small amount of blood (0.05–0.1 mL) from a limb using a hemolet and make a smear (see below). When parasitemia reaches 20%, blood should be passaged to a new host. (Infectivity of thawed material is low, so the parasites should be passaged several times before being harvested.)

**Making a blood smear**

1. Aseptically transfer 0.05–0.1 mL of freshly-drawn blood into an eppendorf tube.
2. Spin down the eppendorf tube at high speed and aspirate the supernatant.
3. Mix the pellet and place a drop of the suspension on a glass slide. Spread the drop into a thin film with the edge of another glass slide. Air dry for 3 mins. at room temperature.
4. Fix air-dried blood film by rinsing with methyl alcohol. Air dry for 3 mins. at room temperature.
5. Stain blood films in 5% Giemsa solution for 15 mins. Rinse with distilled water, air dry.
6. Observe using light microscopy at 1000X magnification to determine parasitemia of culture.

**Reagents for cryopreservation: Reagents**Glycerolyte medium

Glycerol, 57.00 g

NaC<sub>3</sub>H<sub>5</sub>O<sub>3</sub> (Sodium lactate), 1.60 g

KCl, 30.00 mg

NaH<sub>2</sub>PO<sub>4</sub>, 1.38 g

Glass distilled water, 100.00 mL

Mix well and adjust pH to 6.8 using concentrated NaOH and/or HCl. Autoclave and store at 4°C.

Yaeger's anticoagulant

Sodium citrate, 1.33 g

Citric acid, 0.47 g

Dextrose, 3.00 g

Sodium heparin, 0.20 g

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Glass distilled water, 100.00 mL

Filter sterilize.

**Cryopreservation:**

Only young cells (rings) can be frozen in glycerolyte medium\* because their membranes are more robust.

1. To harvest parasites, inject host with ketamine (0.1–0.2 mL).
2. Exsanguinate via the wing vein using a sterile syringe and Yaeger's anticoagulant, 1 volume anticoagulant to 4 volumes blood.
3. Centrifuge blood for 5 mins. at 1800 rpm in 50 mL centrifuge tube.
4. Aspirate supernatant using sterile Pasteur pipet.
5. Resuspend pellet gently in remaining supernatant.
6. Slowly add 5 volumes of glycerolyte medium to 3 volumes pellet dropwise via a syringe as follows:
  - A. Add the first volume of glycerolyte and allow the tube to stand for 5 mins. at room temperature.
  - B. Add the remaining 4 volumes of glycerolyte and gently agitate.
7. Aliquot mixture into Nunc screw-capped freezing vials and place in a Nalgene 1°C cooling apparatus. Place the apparatus at -80°C overnight and then plunge ampules into liquid nitrogen. (The cooling rate in this apparatus is approximately -1°C/min.).
8. Plunge vials into liquid nitrogen (-196°C) the next day and store in liquid nitrogen or liquid nitrogen vapor.

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**Material Citation**

If use of this material results in a scientific publication, please cite the material in the following manner: *Plasmodium relictum* Grassi and Feletti (ATCC 30141)

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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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## Revision

This information on this document was last updated on 2025-03-15

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