Cultivation of Plasmodium falciparum in serum-free media with growth-promoting factor from bovine plasma (GFS)

Version 2

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Abstract

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Protocol

Step 1.

step 1

The composition of media:

1. Complete culture medium (GFSRPMI):

Mix the following constituents aseptically.

(1) 900ml RPMI1640 (with L-Glutamine, with 25mM HEPES, w/o $NaHCO_3$)*

(2) 100ml GFS: GF21 (Wako Pure Chemical Industries, Osaka, Japan)

(3) 2 g (24 mM) sodium bicarbonate (Invitrogen Ltd.)

(4) 25mg gentamycin (Sigma-Aldrich Corp., St. Lowis, MO, USA)

(5) 20 mg (150 µM) hypoxanthine (Sigma-Aldrich)**

*gibco ref. 22400 (Invitrogen Ltd., Carlsbad, CA, USA)

**1.5 ml aliquot of 10 mM solution is added to 100 ml culture medium when using the medium.

2. Basal medium: Mix the following constituents aseptically.

(1) 1000ml RPMI1640 (with L-Glutamine, with 25mM HEPES, w/o $NaHCO_3$)

(2) 25mg gentamycin (Sigma-Aldrich)

Step 2.

Step 2

Start cultures of *Plasmodium falciparum*

1. Preserve erythrocytes (RBCs) in Alsever's solution* at 4 °C for 3– 30 days.
2. Wash RBCs by centrifugation for 10 min at 800g, admixed with RBCs infected with *Plasmodium falciparum* (PfRBCs), dispense into 24-well culture plates at a hematocrit of 2% (1 ml of suspension/well), and culture in a humidified atmosphere of 5%CO_2, 5% O_2, and 90% N_2 at 37°C.
3. Adjust the parasitemia to 0.1% (for subculture) or 0.3%(for growth tests) by adding uninfected RBCs, and the hematocrit to 2% by adding the appropriate volume of culture medium.

*Alsever's solution:

1. Solubilize the following constituents in 200 ml distilled water.

Autoclave the mixture and add to whole blood or RBCs at 1:1 to 1:4.

(1) Glucose 4.1g

(2) NaCl 0.84g

(3) Na_3 citrate.$2H_2O$ 1.6g

(4) Citric acid 0.11g

Step 3.

step 3

Preparation of highly, synchronized cultures at the ring stage:

The cultures of *Plasmodium falciparum* are synchronized at the ring stage by three successive exposures to 5% D-sorbitol.

1. Expose asynchronous PfRBCs to 5% (w/v) D-sorbitol (Sigma-Aldrich) for 5 min.
2. After washing with basal medium by centrifugation for 6 min at 500g, adjust the parasitemia to

0.8%–1.2% by adding uninfected RBCs, dispense into 24-well culture plates at a hematocrit of 2%, and cultured for 41 h.

3. At 41-h culture, expose the PfRBCs to 5% D-sorbitol (the 2nd exposure), wash, and culture for further 5 h.
4. At the total 46-h culture, expose the PfRBCs to 5% D-sorbitol (the 3rd exposure).
5. After the 3rd sorbitol treatment, remove residual schizonts and cell debris by isopycnic density centrifugation for 10 min at 800g on 63% Percoll PLUS* (GE Healthcare Bio-Science Corp., Tokyo, Japan).

*3.5 ml 90% Percoll PLUS + 1.5 ml PBS

90% Percoll PLUS is prepared by admixing 100% Percoll PLUS (9 parts) with 1.5 M NaCl (10x physiological saline) (1 part).

Note: Periods for growth to the schizont stage are somewhat variable among strains. Culture periods useful for the protocol should be confirmed beforehand.