ATCC

Product Sheet

Plasmodium falciparum (ATCC® 30930™)

Please read this FIRST

Storage Temp.

Frozen Cultures:
-70°C for 1 week; liquid N_2 vapor for long term storage

Freeze-dried Cultures:
2-8°C

Live Cultures:
See Protocols section for handling information

Biosafety Level
2

Intended Use

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

Citation of Strain

If use of this culture results in a scientific publication, it should be cited in that manuscript in the following manner: *Plasmodium falciparum* (ATCC® 30930™)

American Type Culture Collection
PO Box 1549
Manassas, VA 20108 USA
www.atcc.org

800.638.6597 or 703.365.2700
Fax: 703.365.2750
Email: Tech@atcc.org

Or contact your local distributor

Description

Strain Designation: FCR-1/FVO
Deposited Name: *Plasmodium falciparum* Welch
Depositor: W Trager
Isolation: Adult human male, Vietnam, 1966 (?)

Propagation

Growth Conditions
Temperature: 37°C
Culture System: *In vitro* culture in human erythrocytes

Medium
ATCC® Medium 2196: Malaria medium, complete

Instructions for Complete Medium
Medium: ATCC Medium 2196 and type O blood

Protocols

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.
2. Aseptically transfer the contents to a 50 mL sterile conical tube.
3. Slowly add 1 volume (0.1 mL) 12% Sodium Chloride solution dropwise via a 1mL syringe to 5 volumes sample (0.5 mL) and agitate continuously.
4. Allow the mixture to stand for 5 mins. at room temperature.
5. Slowly add 5 mL 1.8% Sodium Chloride dropwise via a larger syringe and allow to stand at room temperature for 2 mins.
6. Add 5 mL of 0.9% Sodium Chloride / 0.2% Glucose solution as in step 5.
7. Centrifuge for 5 min. at 1000 x g, remove supernatant.
8. Wash pellet in 20 mL incomplete medium.
9. Centrifuge for 5 min at 1000 x g, remove supernatant.
10. Resuspend pellet in 8mL complete medium in a T-25 tissue culture flask and gently aerate culture with gas mixture of 5% CO_2, 5% O_2 and 90% N_2 using a sterile, cotton plugged Pasteur pipet. Quickly tighten cap of the flask and place in 37°C incubator.
11. Follow protocol for maintenance of culture. Smear as required to determine parasitemia (see below).

Culture Maintenance
Changing of the culture medium every 24 hours is required for a malaria-infected erythrocyte culture. Add washed, uninfected red blood cells (RBCs) to 1-3% haematocrit, and maintain parasitemia at 2-3% for continuous culture.

1. Remove flask with infected culture from 37°C incubator and place onto flask warmer in biological safety hood.
2. Carefully aspirate the medium with a sterile unplugged Pasteur pipet attached to a vacuum line. Remove as much fluid as possible without taking the cells.
3. Aseptically add sterile warm (37°C) completed medium to the flask (~8 mL to a T-25, ~25 mL to a T-75, etc.). Mix and smear as required to determine parasitemia (see below).
4. Add washed RBCs as necessary to obtain desired haematocrit and parasitemia.
5. Gently mix and aerate culture with gas mixture of 5% CO_2, 5% O_2 and 90% N_2 using a sterile, cotton plugged Pasteur pipet. Quickly tighten cap of the flask and place in 37°C incubator until the next medium change.

Making a Blood Smear

1. Aseptically transfer 0.5–1.0 mL of mixed culture with a sterile pipet into a microcentrifuge tube.
2. Spin the microcentrifuge 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

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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.

Cryopreservation

Harvest and Preservation

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

1. Centrifuge ring-stage culture for 5 mins. at 1000 x g in 50 ml centrifuge tube.
2. Aspirate supernatant using sterile Pasteur pipet.
3. Resuspend pellet gently in remaining supernatant.
4. 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.
5. 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.).
6. Plunge vials into liquid nitrogen (-196°C) the next day and store in liquid nitrogen or liquid nitrogen vapor.

References

References and other information relating to this product are available online at www.atcc.org.

Biosafety Level: 2

Appropriate safety procedures should always be used with this material. Laboratory safety is discussed in the current publication of the *Biosafety in Microbiological and Biomedical Laboratories* from the U.S. Department of Health and Human Services Centers for Disease Control and Prevention and National Institutes for Health.

ATCC Warranty

The viability of ATCC® products is warranted for 30 days from the date of shipment, and is valid only if the product is stored and cultured according to the information included on this product information sheet. ATCC lists the media formulation that has been found to be effective for this strain. While other, unspecified media may also produce satisfactory results, a change in media or the absence of an additive from the ATCC recommended media may affect recovery, growth and/or function of this strain. If an alternative medium formulation is used, the ATCC warranty for viability is no longer valid.

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Please see the enclosed Material Transfer Agreement (MTA) for further details regarding the use of this product. The MTA is also available on our Web site at www.atcc.org

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Additional information on this culture is available on the ATCC web site at www.atcc.org.

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