Plasmodium falciparum Welch

50005™

Description

Strain designation: FCR-3/Gambia Subline F-86
Deposited As: *Plasmodium falciparum* Welch
Type strain: No

Storage Conditions

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

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.

BSL 2

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 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.

Certificate of Analysis

For batch-specific test results, refer to the applicable certificate of analysis that can be found at www.atcc.org.

Growth Conditions

Host:

In vitro culture in human erythrocyte

Medium:
ATCC Medium 2196: Malaria medium, complete
Instructions for complete medium: ATCC Medium 2196 and type O blood

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.
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% CO2, 5% O2 and 90% N2 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 (~8ml to a T-25, ~25ml 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 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:

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 1C 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.

Material Citation

If use of this material results in a scientific publication, please cite the material in the following manner: *Plasmodium falciparum* Welch (ATCC 50005)

References

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

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