Plasmodium falciparum Welch

50110™

Description

Strain designation: HB-2
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
Temperature: 37°C

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 1500rpm, remove supernatant.
8. Wash pellet in 20 ml incomplete medium.
9. Centrifuge for 5 min at 1500 rpm, remove supernatant.
10. Resuspend pellet in 8ml complete medium in 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.
11. Smear if required (see below).

*** Medium Formulation:**

To a 500 ml bottle of RPMI-1640 (with $NaHCO_3$, without L-glutamine), aseptically add 18.75ml HEPES (final = 37.5mM), 5ml L-glutamine, 0.25ml Gentamicin, 5ml 20% Glucose (final = 20mM), 3ml 1M Sodium Hydroxide (pH > 7), 5ml Hypoxanthine 100x . Mix thoroughly and remove 100ml from the bottle to make incomplete medium; then add 50ml of heat inactivated human serum (any type) to the remainder to make complete medium (10% serum). Store at 4°C.

NOTE: Outdated blood and serum cannot be used to cultivate this strain. Use only fresh blood and serum.

Culture maintenance: Changing of the culture medium every 24 hours is required for

a malaria-infected erythrocyte 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. Add 25 ml of sterile warm (37°C) complete medium to the flask, gently mix and aerate, then quickly tighten cap of the flask and place in 37°C incubator until next medium change.

Making a Blood Smear:

1. Aseptically transfer 0.5–1ml of mixed culture with a sterile pipet 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 100X 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 min at 1800rpm 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 (see below) 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 screwtop freezing vials and store at -80°C overnight.
6. Plunge vials into liquid nitrogen (-196°C) the next day and store in liquid

nitrogen or liquid nitrogen vapor.

** To formulate glycerolyte medium, combine the following with distilled water to 100 ml: 57.00g glycerol, 1.60g sodium lactate ($C_3H_5NaO_3$), 30.00mg potassium chloride (KCl), 1.38g sodium dihydrogen phosphate (NaH_2PO_4). Mix well and adjust pH to 6.8 using concentrated NaOH and/or HCl. Autoclave to sterilize, and store at 4°C.

Material Citation

If use of this material results in a scientific publication, please cite the material in the following manner: *Plasmodium falciparum* Welch (ATCC 50110)

References

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

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Revision

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Contact Information

ATCC
10801 University Boulevard
Manassas, VA 20110-2209
USA
US telephone: 800-638-6597
Worldwide telephone: +1-703-365-2700
Email: tech@atcc.org or contact your local distributor