Plasmodium berghei Vincke and Lips

50182™

Description

Strain designation: NK65D-CLBB13
Deposited As: *Plasmodium berghei* Vincke and Lips
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 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.

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 vivo, mouse
Instructions for complete medium: *in-vivo* cultivation in mouse
Culture system: Axenic

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 ampules 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 sterile syringe and inject into intraperitoneal cavity 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 (0.05–0.1ml) from a limb using a hemolet and make a smear (see below). When parasitemia reaches 10–30%, parasites should be harvested.

Making a Blood Smear:

1. Aseptically transfer 0.05–0.1ml 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.

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. Open chest cavity to expose heart and exsanguinate via cardiac puncture using Yaeger's anticoagulant** (see below), 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.

Material Citation

If use of this material results in a scientific publication, please cite the material in the following manner: *Plasmodium berghei* Vincke and Lips (ATCC 50182)

References

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

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