Strain Designation: NK65D
Deposited Name: Plasmodium berghei Vincke and Lips
Depositor: NE Alger
Isolation: derived from M. Yoeli strain NK65 by mosquito passage, Univ. Illinois, Urbana, pre-1969

Instructions for Complete Medium

in-vivo cultivation in mouse

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 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.

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.

References

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

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.
Product Sheet

Plasmodium berghei
(ATCC® 50181™)

Please read this FIRST

Storage Temp.
Frozen: -70°C or colder for 1 week, vapor phase of liquid nitrogen for long-term storage

Biosafety Level
1

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 berghei (ATCC® 50181™)

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

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