ATCC

Product Sheet

Babesia microti (ATCC® 30221™)

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: *Babesia microti* (ATCC® 30221™)

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: Gray
Deposited Name: *Babesia microti* (Franca) Reichenow
Depositor: JP Kreier
Isolation: Human, Nantucket Island, MA, 1970

Propagation

Growth Conditions
Culture System: *in vivo*, Golden Syrian hamster

Protocols

Storage and Culture Initiation

NOTE: See the protocol for maintenance *in vivo* in the next section prior to thawing the material.

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.

1. To thaw a frozen ampule, place it in a 35°C water bath such that the lip of the ampule remains above the water line. Thawing time is approximately 2 to 3 minutes. Do not agitate the ampule. Do not leave ampule in water bath after it is thawed.
2. Immediately after thawing, aseptically remove the contents of the ampule with a syringe and inoculate an uninfected hamster. Follow the protocol for maintenance *in vivo*. The course of infection may depend on the recovery of the parasite from the frozen state and the immune status of the host prior to infection.

Culture Maintenance

Yaeger's Anticoagulant
Sodium citrate, 1.33 g
Citric acid, 0.47 g
Dextrose, 3.00 g
Sodium heparin, 0.20 g
Glass distilled H_2O to 100.00 mL

NOTE: Immunosupression of the hamster host is recommended in order to obtain higher levels of parasitemia.

1. Immunosupress a hamster by intraperitoneal injection of cortisone (2mg/day/hamster) or cyclophosphamide (100mg/kg) 1-3 days prior to inoculation.
2. Inoculate entire infected blood suspension intraperitoneally into the hamster using a 1.0 mL syringe equipped with a 27 gauge 1/2 inch needle.
3. Monitor the infection daily or at 2 day intervals by examination of blood films stained with 5% Giemsa solution.
4. Count the number of infected red blood cells (rbc) versus the total number of red cells under oil immersion and determine the % parasitemia: % parasitemia = infected rbc / rbc X 100 A minimum of 500 red blood cells should be counted. (Note that a red blood cell infected with multiple parasites is counted as a single infected cell.)
5. When the level of parasitemia is ≥ 10% the strain should be passaged. Normally, this occurs 2-3 weeks post-inoculation of an immunosupressed hamster and over one month in an immunocompetent hamster. The level of parasitemia before death will vary with the strain and immune status of the hamster used. Monitoring on a daily basis will alert the experimenter as to when the parasite should be passaged.
6. To passage the parasite, remove blood from the infected hamster using cardiac puncture using a syringe and suitable anticoagulant:
   a. In a laminar flow hood ventilated to the outside, add one capful of the Metofane (Pitman-Moore, Inc. Washington Cross, NJ, cat# 55685) to a wad of cotton at the bottom of a gallon jar. Place a wire mesh screen over the top of the cotton and tightly secure the lid. Allow the jar to remain undisturbed for 10 minutes. Remove the lid of the jar and add the infected hamster. When the animal is thoroughly anesthetized, tie it down firmLy with its stomach upward. Thoroughly swab the chest with 70% denatured alcohol.
   b. Add 0.1 mL of anticoagulant solution (Yaeger's or heparin, etc.) to a 1.0 mL syringe equipped

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with a 27 gauge 1/2 inch needle. Puncture the heart and move the plunger of the syringe back and forth several times to distribute the anticoagulant.

c. Draw blood into the syringe by gently pulling the plunger outward. When blood is no longer obtainable or the hamster has died, remove the needle from the animal and invert the syringe several times to mix the anticoagulant evenly with the blood.

d. Remove air bubbles from the syringe. Place the syringe in a vertical position with the needle pointing upward. Place the tip of the needle on the surface of a cotton ball thoroughly wet with alcohol (squeeze the cotton ball so that it is moist but not dripping wet). With the index finger flick the top of the syringe several times to allow the air bubbles to coalesce and move to the top of the syringe body. Gently push in the plunger to remove the air pocket. It may be necessary to repeat this procedure several times to remove all the air bubbles. When a steady stream of blood exits the needle, the blood is ready for injection.

7. Inject 0.2 mL of the infected blood suspension into each uninfected hamster.
8. Monitor parasitemia and passage as needed.

Cryopreservation

Reagents

Alsever's Solution
NaCl, 4.2 g
Na_3citrate•$2H_2O$, 8.0 g
Glucose, 20.5 g
Glass distilled H_2O to 1.0 L
*Dissolve components in glass distilled H_2O, adjust the pH to 6.1 with 10% (w/v) citric acid and filter sterilize. The solution can be obtained from Sigma-Aldrich (cat# A3551).

Harvest and Preservation

1. Prepare a 30% (v/v) sterile glycerol solution in Alsever's solution.
2. Draw approximately 0.1 mL of anticoagulant solution (Yaeger's or heparin, etc.) into a syringe and move it back and forth over the length of the syringe, several times. Remove all air bubbles. Draw blood by cardiac puncture into the syringe from a host animal that has reached or is near peak parasitemia. If clotting occurs during extraction of blood, insufficient heparin was used.
3. Mix the heparinized blood with the 30% glycerol solution in a 2:1 ratio. If any clotting has occurred do not use. After mixing, the final concentration of cryoprotectant solution will be 10% (v/v). The mixture should be placed in a 4°C ice bath. The time from the mixing of the cell preparation and glycerol stock solution before the freezing process is begun should be no less than 15 min and no longer than 30 min.
4. Dispense in 0.5 mL aliquots into 1.0 - 2.0 mL sterile plastic screw-capped cryules (special plastic vials for cryopreservation). Filled ampules should be placed in a 4°C ice bath. Do not immerse ampules to the level of the vial cap.
5. Plunge ampules from 4°C into liquid nitrogen. The frozen preparations may be stored in a mechanical freezer until needed, however storage in either the vapor or liquid phase of a nitrogen refrigerator is recommended for the longest viability.
6. To thaw a frozen ampule, place in a 35°C water bath, until thawed (2-3 min). Immerse the ampule just sufficient to cover the frozen material. Do not agitate the ampule.
7. Immediately after thawing, aseptically remove the contents of the ampule with a syringe and inoculate an uninfected hamster. Follow the protocol for maintenance *in vivo*. The course of infection may depend on the recovery of the parasite from the frozen state and the immune status of the host prior to infection.

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

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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.

Disclaimers

This product is intended for laboratory research purposes only. It is not intended for use in humans.

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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

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

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