Product Information Sheet for NR-21971

Bulinus truncatus subsp. *truncatus* (Unexposed to *Schistosoma haematobium*)

Catalog No. NR-21971

For research use only. Not for human use.

Contributor and Manufacturer:
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Product Description:
Species: *Bulinus truncatus* subsp. *truncatus*
Original Source: The *Bulinus truncatus* (*B. truncatus*) subsp. *truncatus* snails are from an unknown location in Egypt.[1]

B. truncatus subsp. *truncatus* is a species of air-breathing freshwater snail. It is found in North Africa and the Middle East, where it is an intermediate host of the *Schistosoma haematobium* (*S. haematobium*) trematode worm parasite. *S. haematobium* infection causes schistosomiasis in humans.

Material Provided:
Up to 100 *B. truncatus* subsp. *truncatus* in a Petri dish.[1]

Packaging/Storage:
NR-21971 is packed in moist paper towels in a Petri dish encased in bubble-wrap and shipped overnight in cardboard boxes (42.5 cm x 23 cm x 23 cm). To insure viability, snails should be placed in suitable aquaria at 26°C to 28°C.

Growth Conditions:[2,3]
Food Source:
Romaine lettuce or *Nostoc* blue-green algae grown on a layer of autoclaved mud may be used as food sources (see Appendix I for details).
Maintenance of *B. truncatus*:
Depending on the number of snails to be maintained, they may be kept in plastic trays (approximately 1.5 L volume) or larger aquaria (40 L to 110 L) in filtered tap water that has been aerated for 2 to 3 days (pH ~ 7.1). The ambient temperature should be 26°C to 28°C, and the aquaria should be under continuous illumination from a 40-watt fluorescent bulb.
Propagation of *B. truncatus*:
1. Place ten snails in plastic trays containing aerated tap water and Styrofoam (75 mm x 125 mm). Maintain trays at 26°C to 28°C under continuous illumination.
2. At the end of one week, remove egg masses from the trays and Styrofoam and transfer to Petri dishes that contain aerated tap water.
3. After one week, 50 newborn snails 0.6 mm to 0.8 mm in shell diameter are transferred with a pipette to each Petri dish that contains water, mud and blue-green algae.
4. Each week, transfer and maintain snails at a density of 25 snails per dish. After 14 days, 3 mm to 5 mm shell diameter snails may be infected with *S. haematobium* or used to initiate new cultures.

Exposing Snails to Miracidia:[4]
Large numbers of miracidia can be obtained from the intestinal walls of hamsters infected for 4 months with 400 *S. haematobium* cercariae per hamster.
1. Mince tissue containing eggs for 30 seconds in 0.85% NaCl, using a low speed setting on a Waring blender.
2. Centrifuge homogenate for 5 minutes at 100 x g.
3. Pour off the supernatant and resuspend the pellet in aged tap water that has been pre-warmed to 26°C.
4. Place the egg suspension in a 1 L darkened side arm flask in which the side arm is not darkened and fill the flask with aged tap water.
5. Shine a light on the exposed side arm, taking care not to overheat the side arm. Since the miracidia are phototropic they will begin to collect in the water of the side arm in 20 to 30 minutes, at which point they can be removed by a Pasteur pipette.
6. Withdraw a pipette full of miracidial suspension, and place it in a Petri dish with additional aged tap water.
7. Add aged tap water back into the side-arm flask to keep the volume constant.
8. With a drawn Pasteur pipette and using a dissecting microscope, withdraw the appropriate number of miracidia and place with the snails in a small volume of water.
9. Incubate snails with miracidia for at least 2 hours to ensure miracidial penetration.

Citation:
Acknowledgment for publications should read "The following reagent was obtained through BEI Resources, NIAID, NIH: *Bulinus truncatus* subsp. *truncatus* (Unexposed to *Schistosoma haematobium*), NR-21971."

Biosafety Level: 1
Appropriate safety procedures should always be used with this material. Laboratory safety is discussed in the following publication: U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control and Prevention, and National Institutes of Health. Biosafety in Microbiological and Biomedical Laboratories. 5th ed. Washington, DC: U.S. Government Printing Office, 2007; see www.cdc.gov/od/ohs/biosfty/bmbl5/bmbl5toc.htm.

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References:

1. F. A. Lewis, Personal Communication.
2. Lewis, F. "Schistosomiasis." Curr. Protoc. Immunol. Chapter 19 (2001): Unit 19.1. PubMed: 18432750.
3. Bruce, J. I. and Y.-S. Liang. "Cultivation of Schistosomes and Snails for Researchers in the United States of America and Other Countries." J. Med. Appl. Malacol. 4 (1992): 13-30.
4. Lewis, F. A. et al. "Large-Scale Laboratory Maintenance of *Schistosoma mansoni*, with Observations on Three Schistosome/Snail Host Combinations." J. Parasitol. 72 (1986): 813-829. PubMed: 3546654.

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APPENDIX I: PREPARATION OF MUD-BASED MEDIA FOR GROWTH OF *NOSTOC* ALGAE[3]

Equipment
Autoclave
Spatula(s)

Materials
Mud or soil source
Chicken manure
Calcium carbonate (pulverized limestone)
Clay
0.06% sodium nitrate solution prepared with aged tap water
Nostoc (stock cultures can be obtained from Ward's Biological Supply, Rochester, NY)
Plastic Petri dishes (25 mm x 100 mm)
Stainless steel baking pan (250 mm x 380 mm x 75 mm)

Procedure
1. The proportions of dried mud, lime and chicken manure needed for good growth of *Nostoc* will likely vary, depending on the richness of the soil obtained. A small amount of clay may be necessary for cohesion of the mud mound that will be placed in the Petri dishes. The following describes the current proportions of each component for the soil. Trial and error will be the rule, rather than the exception, to accommodate the apparent richness (or lack thereof) of soils in different regions.
2. The soil and site chosen ideally should be one where there is considerable sedimentation (e.g. a stream bed bottom) or topsoil. Soil should be obtained where no known herbicides or pesticides have been used.
3. The mud or soil brought back to the laboratory from the field site should be strained through a series of crude screens to remove rocks and other large debris. Once it is a fine consistency, it should be completely dried before use.
4. Mix 3 kilograms of dried mud with 90 grams lime (pulverized limestone) and 30 grams dried chicken manure. To this mixture add enough tap water to make a paste. Place the mud mixture in a large stainless steel baking pan and cover with aluminum foil. The depth of the mud should be no more than about 100 mm. Autoclave for a continuous 2 hours.
5. Once the mud is autoclaved and cooled to room temperature, use a sterile spatula (spatulas should be wiped down periodically with gauze drenched in pure alcohol) and place about 40 grams of the still wet mud in the center of a Petri dish and form a smooth and solid mud mound about 15 mm high and 60 mm in diameter. If the mud has dried too much during autoclaving and needs some additional liquid to make it easier to spread, add a few mL of sterile 0.06% nitrate solution and mix thoroughly. To expedite the spreading process, one can use two curved sterile spatulas to stir a third to half of the mud in the steel pan (adding the sterile 0.06% nitrate solution as needed) before spreading it into the Petri dishes. This ensures consistency of the ingredients in the mud that is placed in each Petri dish.
6. Once the mud mounds have been formed in the Petri dishes, cover the mud mound with 0.06% nitrate solution and add about 2 mL of a suspension of *Nostoc* (in 0.06% nitrate solution) to seed the plate for new growth. Be sure not to flood the Petri dish with liquid, so that the lid does not become wet with the growth medium.
7. Cover and place under fluorescent lighting (40 watt, cool-white fluorescent) at 25°C to 27°C for 1 to 3 weeks. For best results the lights should be about 30 cm above the Petri dishes.
8. The preparation is suitable for feeding to the snails once a solid mat of the *Nostoc* has grown over the surface.