Oncomelania hupensis subsp. *quadrasi*, Philippine Strain (Unexposed to *Schistosoma japonicum*)

Catalog No. NR-32834

For research use only. Not for human use.

Contributor and Manufacturer:

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Product Description:

Species: *Oncomelania hupensis* subsp. *quadrasi*

Original Source: The *Oncomelania hupensis (O. hupensis)* subsp. *quadrasi*, Philippine strain, snail host originates from Leyte, Philippines.[1]

O. hupensis subsp. *quadrasi* is a species of fresh water snail found in in China and the Far East. It is an intermediate host of the *Schistosoma japonicum* (*S. japonicum*) trematode worm parasite. *S. japonicum* infection causes schistosomiasis in China and in areas with poor sanitation that lack access to safe drinking water.[2]

Material Provided:

NR-32834 consists of up to 100 *O. hupensis* subsp. *quadrasi,* Philippine strain, in a Petri dish.[1]

Packaging/Storage:

NR-32834 is packaged 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. Note: Infected snails are exposed to miracidia only a few days before shipment. Laboratories should be aware and take caution of possible cercarial exposure following a 4 week prepatent period after exposure to miracidia (depending on the temperature and species). BEI Resources recommends consulting personnel in your institution's Occupational Safety and Health office for clearance to receive and maintain the infected snails.

Growth Conditions:[3,4]

Food Source:

Nostoc blue-green algae grown on a layer of autoclaved mud (see Appendix I for details) is used as a food source.

Maintenance of *O. hupensis* snails:

Snails may be kept in covered shallow trays (approximately 25 to 50 mm deep) in filtered tap water that has been aerated for 2 to 3 days (pH ~ 7.1). The ambient temperature should be 25°C to 26°C and the snails should be maintained under continuous illumination. Note: *Oncomelania* snails are amphibious, so many will crawl onto the underside of the lid and remain there. Hanging snails may be removed and placed back into water when the container is changed.

Propagation of *O. hupensis*:

1. Identify male and female *O. hupensis* snails by individually examining fully-grown (8 mm) snails in a Petri dish. Place the adult snail in a horizontal position by inserting the shell apex into modeling clay affixed to the rim of the Petri dish.
2. Flood Petri dish with water. When head of the snail extends, examine under a dissecting microscope. Male snails can be identified by the presence of a verge (penis) which is visible between the mantle collar and neck of snail but may not extend past the shell opening.
3. Add 20 to 30 breeding pairs (1 male, 1 female) of snails to a shallow (25 mm deep) tray containing algae/mud food source and 25 mm of water.
4. Change the water every 10 to 14 days by pouring contents of tray through 0.5 mm sieve. Rinse container and resuspend mud layer by washing with a stream of water. Pass resuspended mud through sieve.
5. Place adult snails in new container containing algae/mud and aged aerated tap water. *O. hupensis* should start to lay eggs within 2 to 3 months of breeding. Eggs appear as small soil-colored specks about 1 mm in diameter.
6. Eggs attached to the bottom of the container can be gently dislodged with a spatula and transferred with a glass Pasteur pipette to a Petri dish containing aged tap water.
7. Eggs deposited in the mud can be identified by placing the sieved material in a Petri dish with aged tap water and gently agitating it. Eggs are oval in shape and move more readily than other filtered material. Use a glass Pasteur pipette to transfer these eggs to a Petri dish containing aged aerated tap water. Eggs hatch approximately 16 days after they are laid. Check dish daily under dissecting microscope for juvenile snails, which are very small and transparent. Transfer juveniles to Petri dish containing a small amount of algae to prevent overgrowth.
8. 1 to 2 weeks later, transfer these juveniles to a fresh dish. Under optimal conditions, *O. hupensis* snails should reach adulthood 1 to 2 months after hatching.

Collecting Cercariae:

1. Observe infected snails for the presence of secondary sporocysts in the apex area of the shell.
2. Place a few infected snails on an inverted Petri dish top and gently crush the snails with the bottom of the dish. Add a few drop of water to the crushed snails and dissect out the secondary sporocysts.
3. Transfer sporocysts to a 60 mm Petri dish that contains aged, aerated tap water. Tease apart sporocysts with fine forceps and a small gauge needle to release cercariae. The most infective cercariae will be those that swim to the top of the water and remain there. Each infected snail can yield 300 to 500 cercariae. To ensure mixed sex infections of mice, obtain cercariae from at least 10 snails.

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Acknowledgment for publications should read "The following reagent was obtained through BEI Resources, NIAID, NIH: *Oncomelania hupensis* subsp. *quadrasi*, Philippine Strain (Unexposed to *Schistosoma japonicum*), NR-32834."

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, 2009; see http://www.cdc.gov/biosafety/publications/bmbl5/index.htm.

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References:

1. M. Tucker, Personal Communication.
2. Roberts, L. and G. D. Schmidt. Foundations of Parasitology, 7th ed. Dubuque, Iowa: Wm. C. Brown Publishers, 2005.
3. Bruce, J. I., M. G. Radke and G. M. Davis. "Culturing *Biomphalaria* and *Oncomelania* (Gastropoda) for Large-Scale Studies of Schistosomiasis." Biomedical Report No. 19, 406th Medical Laboratory. (1971) U. S. Army.
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APPENDIX I: PREPARATION OF MUD-BASED MEDIA FOR GROWTH OF *NOSTOC* ALGAE[5]

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.