**Schistosoma mansoni**, Strain NMRI
Exposed *Biomphalaria glabrata*, Strain NMRI

**Catalog No. NR-21962**

For research use only. Not for human use.

**Contributor and Manufacturer:**
Fred A. Lewis, Head Schistosomiasis Laboratory, Biomedical Research Institute, Rockville, MD (NIH-NIAID Contract HHSN272201000005I)

**Product Description:**
- **Flatworm Classification:** Schistosomatidae, Schistosoma
- **Species:** *Schistosoma mansoni*
- **Strain:** NMRI
- **Original Source:** *Schistosoma mansoni* (S. mansoni), strain NMRI was isolated in the 1940s from *S. mansoni* eggs obtained from infected Puerto Rican school children.\(^1\)
- **Host:** The *Biomphalaria glabrata* (B. glabrata), strain NMRI snail host is an albino snail that is highly susceptible to the NMRI strain of *S. mansoni*. It was derived from a cross between pigmented Puerto Rican (susceptible) snails and Newton’s M-line snails.\(^1\)
- **Comment:** *S. mansoni*, strain NMRI was brought to the Naval Medical Research Institute (NMRI) in 1945, where it was maintained until 1971, at which time it was transferred to the Biomedical Research Institute.\(^1\)

*S. mansoni* is a species of trematode worm which causes the chronic parasitic disease Schistosomiasis. Worldwide, more than 200 million people are infected and nearly 700 million are at risk, primarily in areas with poor sanitation that lack access to safe drinking water.\(^2\)

Infection occurs through contact with larval-stage schistosomes ( cercariae) that are released by freshwater snails. Upon exposure to infested water, these larvae penetrate human skin and travel through blood vessels to the liver where they mature and deposit eggs. Some of these eggs are then passed through human feces into water to re-infest the snail host and continue the parasite’s life cycle. Schistosome eggs that remain in the human body cause an immune response and damage to internal organs.\(^3\)

**Material Provided:**
NR-21962 consists of up to 100 *B. glabrata*, strain NMRI, infected with *S. mansoni*, strain NMRI, in a petri dish.\(^1\)

**Packaging/Storage:**
NR-21962 is packed in moist paper towels in a Petri dish encased in bubble-wrap and shipped overnight in cardboard boxes (425 mm x 225 mm x 225 mm). 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:**\(^4,5\)
**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. glabrata*:**
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 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. glabrata*:**
1. Place ten snails in plastic trays containing aerated tap water and Styrofoam (75 mm x 120 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. mansoni* or used to initiate new cultures.

**Collecting Cercariae:**
1. Screen infected snails individually under a dissecting microscope in a Petri dish containing a small volume of aerated tap water for the presence of *Schistosoma* sporocysts, which appear as light colored flecks that are visible through the shell.
2. Place infected (patent) snails in a beaker where moisture is maintained but does not contain water. Let beaker stand for 15 to 30 minutes at 26°C to 28°C under ceiling illumination.
3. Rinse snails with a small volume of water. Add a second volume of water (100 mL per 100 snails). Place uncovered beaker under a 15-watt white fluorescent light, 18 inches above the beaker for 20 minutes. Decant water containing cercariae into another beaker. On average, 2000 to 4000 cercariae may be obtained from each infected snail.
4. To estimate the cercarial density, stir the beaker that contains cercariae continuously with a magnetic stir bar, and remove 0.1 mL aliquots from the center of the suspension.
5. Place each aliquot in a 10-cell depression slide. Add

**BEI Resources**
[www.beiresources.org](http://www.beiresources.org)

© 2012 American Type Culture Collection (ATCC). All rights reserved.

E-mail: contact@beiresources.org
Tel: 800-359-7370
Fax: 703-365-2898
one drop of Lugol’s Iodine solution to each cell prior to counting under a dissecting microscope. Determine the cercariae density per volume. This suspension may subsequently be diluted, or concentrated by pipetting out the excess liquid.

Citation:
Acknowledgment for publications should read “The following reagent was obtained through BEI Resources, NIAID, NIH: *Schistosoma mansoni*, Strain NMRI Exposed *Biomphalaria glabrata*, Strain NMRI, NR-21962.”

Biosafety Level: 2

Disclaimers:
You are authorized to use this product for research use only. It is not intended for human use.

Use of this product is subject to the terms and conditions of the BEI Resources Material Transfer Agreement (MTA). The MTA is available on our Web site at www.beiresources.org.

While BEI Resources uses reasonable efforts to include accurate and up-to-date information on this product sheet, neither ATCC® nor the U.S. Government makes any warranties or representations as to its accuracy. Citations from scientific literature and patents are provided for informational purposes only. Neither ATCC® nor the U.S. Government warrants that such information has been confirmed to be accurate.

This product is sent with the condition that you are responsible for its safe storage, handling, use and disposal. ATCC® and the U.S. Government are not liable for any damages or injuries arising from receipt and/or use of this product. While reasonable effort is made to ensure authenticity and reliability of materials on deposit, the U.S. Government, ATCC®, their suppliers and contributors to BEI Resources are not liable for damages arising from the misidentification or misrepresentation of products.

Use Restrictions:
This material is distributed for internal research, non-commercial purposes only. This material, its product or its derivatives may not be distributed to third parties. Except as performed under a U.S. Government contract, individuals contemplating commercial use of the material, its products or its derivatives must contact the contributor to determine if a license is required. U.S. Government contractors may need a license before first commercial sale.

References:
1. F. A. Lewis, Personal Communication.

ATCC® is a trademark of the American Type Culture Collection.
APPENDIX I: PREPARATION OF MUD-BASED MEDIA FOR GROWTH OF NOSTOC ALGAE

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)

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 1 foot 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.