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Infection of *Biomphalaria glabrata* snails with *Schistosoma mansoni* miracidia

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Schistosoma mansoni



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We use this protocol and it's working

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ABSTRACT

To infect *Biomphalaria glabrata* snails with miracidia hatched from *Schistosoma mansoni* eggs

MATERIALS

☒ MilliQ water **Contributed by users**

☒ 24-well Clear TC-treated Multiple Well Plates Individually Wrapped Sterile **Costar Catalog #3524**

☒ Blunt featherweight forceps wide tip **BioQuip Catalog #4750**

☒ 1x DPBS **Gibco - Thermo Fischer Catalog #14190144**

☒ Glass Pasteur pipettes with rubber bulbs **Contributed by users**

1x Aquarium Water (diluted from 10X; see recipes)

1-2L flask

Aluminum foil

B. glabrata snails 5-8mm

S. mansoni infected livers in PBS

28°C room or incubator

Incubator at 37°C and 5% CO₂

Bucket of ice

Equipment

Zoom stereomicroscope with diascope illumination stand

NAME

stereomicroscope

TYPE

Nikon

BRAND

SMZ800N

SKU

10X AQUARIUM WATER

5.56g CaCl₂

12.28g MgSO₄·7H₂O

0.43g K₂SO₄

4.2g NaHCO₃

480µl FeCl₃·6H₂O (0.5g/100ml water)

Fill to 10L and store in 1L bottles

Dilute to 1x: 9L MQ water + 1L 10x

Lung preparation

1

Collect lungs from patent mice into 50ml Falcon tubes containing pre-warmed 37°C 1x DPBS

- 2 Remove lungs from PBS and place in large mortar or laboratory blender
- 3 Gently homogenise lung tissue
- 4 Place homogenised lung slurry into a flask (size of flask depends on how many lungs you have) and fill with diH₂O or aquarium water

Hatching miracidia

- 5 Cover flask in aluminum foil and shine light horizontally across the opening of the flask for 1-2 hrs
- 6 Take a small aliquot of water from the top of the flask using a glass Pasteur pipette and place into a petri dish. Using a stereomicroscope, check for swimming miracidia

Exposing snails to miracidia for regular life cycle mainten.

- 7 Under a microscope, collect 15 miracidia with a glass Pasteur pipette per well

Alternatively (but less preferred) estimate the number of miracidia by counting 12-5µl aliquots in a petri dish. After collecting (shaking the tube in between) and placing the aliquots in the petri dish add 5µl of Lugol to each drop (this kills and stains the miracidia)
- 8 Place individual 5-8mm snails into 24-well culture plates and cover snails completely with 1x aquarium water

- 9 Expose snails to miracidia for at least 3 hours (up to overnight)
- 10 After exposure, remove the snails carefully using featherweight forceps and put them in a new tank with food

Exposing snails to miracidia for monomiracidium infections

- 11 Dilute miracidia so that one miracidium can easily be collected in ~3-5µl of water
- 12 Collected single miracidium using fresh 10µl pipette each collection and place in 24-well plate
- 13 After a plate is filled, check each well under a microscope to verify there is a single miracidium in each well
- 14 Place individual 5-8mm snails in the wells containing confirmed single miracidium and cover snails completely with 1x aquarium water
- 15 Leave the plate overnight (inside incubator or room at 28°C)
- 16 The following day, transfer the snails to a new tank with food

