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Infection of Biomphalaria glabrata snails with Schistosoma mansoni miracidia V.2

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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

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 Fisher Scientific Catalog #**14190144

Glass Pasteur pipettes with rubber bulbs

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 diascopic 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

Troubleshooting

Liver preparation

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

2 Remove livers from PBS and place in large mortar or laboratory blender

3 Gently homogenise liver tissue

4 Place homogenised liver slurry into a flask (size of flask depends on how many livers you have) and fill with diH_2O 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 maintenance

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