Isolation of Schistosoma mansoni eggs, miracidia, and sporocysts for in vitro cultivation

Sarah K Buddenborg[1]

[1]Wellcome Sanger Institute

Schistosoma mansoni

Sarah K Buddenborg
Wellcome Sanger Institute, University of Cambridge

ABSTRACT

The purpose of this procedure is to isolate eggs from livers collected from mice infected with *Schistosoma mansoni*. This protocol ensures a sterile prep of eggs to be used for culture of eggs and/or collection and culture of sporocysts, and snail infection.

GUIDELINES

Livers should be placed on ice, following mouse perfusion. This protocol is for up to 5 livers only. (i.e. for 20 livers 4 tubes containing 5 processed livers are used, and for 18 livers 2 tubes containing 5 livers each plus 2 tubes containing 4 livers each are used).

All steps should be performed in a sterile, cleaned tissue culture hood.

APR 17, 2023

OPEN ACCESS

DOI:
dx.doi.org/10.17504/protocols.io.81wgbye5yvpk/v1

Protocol Citation: Sarah K Buddenborg 2023. Isolation of Schistosoma mansoni eggs, miracidia, and sporocysts for in vitro cultivation. **protocols.io** https://dx.doi.org/10.17504/protocols.io.81wgbye5yvpk/v1

Protocol status: Working
We use this protocol and it's working

Created: Apr 11, 2023

Last Modified: Apr 17, 2023

PROTOCOL integer ID: 80333

Keywords: helminths, in vitro sporocysts, schistosoma mansoni

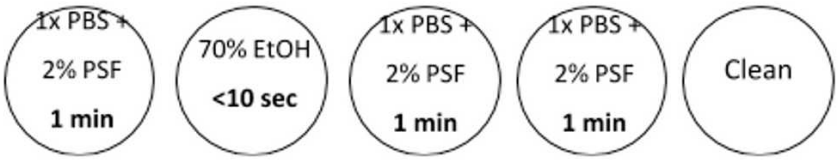
MATERIALS

BIOMAT 2 Class 2 Microbiological Safety Cabinet
Rocking incubator at 37°C
Lamp or other light source
Pipette boy
Tweezers
Parafilm
2ml aspirating pipettes
50ml stripettes
25ml stripettes
250um sterile sieve
150µm sterile sieve
1L sterile beakers (x2)

- Fetal Bovine Serum **Gibco - Thermo Fischer Catalog #10270106**
- DMEM, high glucose, GlutaMAX™ Supplement **Thermo Fisher Catalog #10566032**
- 50ml Falcon tubes **Corning Catalog #352070**
- Falcon™ 15mL Conical Centrifuge Tubes **Fisher Scientific Catalog #14-959-53A**
- 70% Ethanol **Contributed by users**
- 1x DPBS **Gibco - Thermo Fischer Catalog #14190144**
- Collagenase **Merck MilliporeSigma (Sigma-Aldrich) Catalog #C5138**
- MilliQ water **Contributed by users**
- Sucrose **Merck MilliporeSigma (Sigma-Aldrich) Catalog #S7903**
- Percoll **Merck MilliporeSigma (Sigma-Aldrich) Catalog #P1644-500ML**
- Petri dishes sterile **VWR International Catalog #516-8029**
- Swann-Morton Stainless Steel Surgical Scalpels # 21 **Fisher Scientific Catalo #11748353**
- Antibiotic-Antimycotic 9100x0 [Anti-Anti] **Thermo Fisher Scientific Catalog #15240062**

Liver washing

1 In tissue culture hood, prepare three petri dishes with 1x DPBS+2% anti-anti, one with 70% ethanol, and one clean petri dish arranged in the following order:



2 Decant the livers into the first petri dish using ethanol-cleaned tweezers to submerge and continuously move them for 1 minute to ensure complete saturation of the tissue

3 Repeat step 3 for each petri dish with the exception of the 70% ethanol dish which should be submerged and rinsed for less than 10 seconds

Liver dissociation

4 Place all livers into a clean petri dish and using a sterile scalpel finely mince them

5 Transfer the minced livers to a 50ml falcon tube, re-suspend in 40ml of 1x DPBS + 2% anti-anti and label with necessary identifying information

6 Weigh out 0.05g of collagenase into a labelled 15ml falcon tube and add 10ml of dH_20. Mix well (The amount of collagenase to prepare depends on the number of livers to be processed – collagenase is always prepared fresh)

7 Add 5ml of 0.5% collagenase solution to the liver suspension and mix well

- Optional: Add to the mix 500 ul of polymixin B (100K Units) (Sigma-Aldrich, P4932-1MU), a gram negative bactericidal antibiotic that reduce LPS contamination in the egg prep, in particular when SEA will be prepared from eggs and immunological studies or co-culture with cells, will be performed

8 Wrap securely in parafilm and secure the tube horizontally in a 37°C rocker overnight

Egg isolation

9 The following day, centrifuge the liver suspension tube at 400g for 5 min (acceleration and deceleration 9)

10 Aspirate the supernatant and re-suspend in 50ml of 1x DPBS + 2% anti-anti.

11 Repeat steps 9-10 three more times

12 After the final aspiration, re-suspend the pellet in 25ml of 1x DPBS + 2% anti-anti

13 Using a 50ml stripette, pass the suspension through the 250uM sieve into a 1L sterile beaker

14 Pass this filtrate through the 150uM sieve into a second 1L sterile beaker

15 Wash the beaker with 5ml of 1x DPBS + 2% anti-anti to collect any remaining eggs and add this to the filtrate by passing through the 150uM sieve

16 Decant the filtrate into a 50ml falcon tube and centrifuge (400g, 5 minutes, acceleration and deceleration 9)

17 Aspirate the supernatant and resuspend in 10ml 1x DPBS + 2% anti-anti

18 Prepare a Percoll gradient (one percoll gradient per 5 livers):

18.1 Prepare a 0.25M sucrose solution (4.27g sucrose + 50ml diH_2O) and filter the solution through 0.22um syringe filter

18.2 In 50ml falcon tube mix 8ml Percoll and 32ml of the 0.25M sucrose solution. Invert 5 times

19 Very carefully pipette the resuspended eggs onto the surface of the Percoll gradient around the circumference to create a defined layer

20 Centrifuge the gradient at 800g for 10 minutes (acceleration 2 and deceleration 1)

21 Aspirate the supernatant and re-suspend in 10ml of 1x DPBS + 2% anti-anti. Transfer to a 15ml falcon tube

22 Centrifuge at 400g for 5 min (acceleration and deceleration 9)

23 Repeat steps 21-22 two more times

24 ***IMPORTANT. Check the eggs under microscope, if the prep is still 'dirty' or 'contaminated with liver debris' proceed with a second percoll gradient***

25 Resuspend the pellet in 10ml 1x DPBS + 2% anti-anti and count 12 aliquots of 5µl to estimate total number of collected eggs

Eggs in culture

26 Centrifuge eggs in falcon tube at 400g for 5 mins (acceleration and deceleration 9)

27 Resuspend eggs in adult media (DMEM + 10% FBS + 2% anti-anti) and transfer to 6 well plates (5-6ml of media per well)

28 Keep eggs in culture at 37°C and 5% CO_2

- *Eggs can be kept in culture for up to ~10 days and retain the 'hatchability' however, the hatching rate will drop over time*

Hatching eggs and collecting miracidia

29 Centrifuge eggs in falcon tube at 400g for 5 mins (acceleration and deceleration 9)

30 In the culture hood, aspirate supernatant and re-suspend in 6ml diH_2O

31 Aliquot 1ml each in a 24 well plate

- *It is important to use 24 well plate given the miracidia get more diluted in 12 or 6 well plate and more egg shells are picked up when collecting the miracidia)*

32 Rinse the original falcon tube with 6 ml of water and distribute 1 ml to each well containing 1ml of eggs (i.e. the eggs will be in 2ml of water)

33 Place under light for hatching

34 At 30-40 min intervals over ~3 hrs, gently remove the top 1ml of water containing the miracidia into a 50ml falcon tube and top up the wells with 1ml of diH_2O

35 Count miracidia and proceed with snail infections dx.doi.org/10.17504/protocols.io.36wgqjkkxvk5/v1 or sporocyst transformation

Sporocyst transformation

36 Place the tube containing miracidia on ice for ~20 min

37 Centrifuge at 800g for 15min (acceleration and deceleration 9)

38 **Quickly** aspirate the water and resuspend the pellet of miracidia in complete sporocyst media

- Sporocysts can be kept in culture at 28°C with malaria gas (90-92% N, 5% CO_2, 3-5% O_2) in a sealed container changing the media once to twice a week
- **IMPORTANT.** To avoid contamination always replace the media with fresh complete media the day after transformation