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Culturing schistosomula

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Introduction

Infectious cercariae are transformed *in vitro* into schistosomula. Schistosomula can be cultured *in vitro* in a complex medium. These *in vitro* cultured schistosomula do not grow at the same rate as those in a permissive host, nor will they become patent adults, but in our hands about 50% of them will mature with fully formed guts, and 10% will develop into sexually distinct male and female worms. Hundreds of thousands of worms can be easily maintained, providing a vast amount of parasite material, allowing for genetic manipulation through RNAi and transgenesis. The percentage of schistosomula forming guts and growing properly can be increased (50% versus 20%) by supplementing the media with conditioned media during the first week of culture.

Equipment

37 °C/5% CO_2 incubator
Biosafety cabinet/Tissue culture hood

Reagents

Schistosomulum Wash (SW)

500 ml	RPMI (Cellgro, 15-040)
5 ml	Hepes Buffer (Cellgro, 25-060-CI)
10 ml	Antibiotic/Antimycotic (Invitrogen, 15240-062)

Schistosomulum Wash plus Tween (SWAT)

SW + 0.5% Tween 20

Schistosomulum Medium (SM)

20X Lactalbumin hydrolysate/glucose-

2.5 g	lactalbumin hydrolysate (Sigma, L9010)
2.5 g	glucose (Sigma, G5400)
250 ml	Basal Medium Eagle (Gibco, 21010046)

- Filter sterilize
- Store at 4 °C

For 1 liter of SM:

50 ml	20X Lactalbumin hydrolysate/glucose
0.5 ml	Hypoxanthine (1 mM) (-20 °C) (Sigma, H9377)
1 ml	Serotonin (1 mM) (-20 °C) (Sigma, H9523)
1 ml	Insulin (8mg/ml) (4 °C) (Sigma, I0516)

1 ml	Hydrocortisone (1 mM) (-20 °C) (Sigma, H0888)
1 ml	Triiodothyronine (0.2 mM) (-20 °C) (Calbiochem, 64245)
5 ml	MEM Vitamins (100X) (-20 °C) (Invitrogen, 11120-052)
50 ml	Schneiders Medium (Drosophila) (+4 °C) (Invitrogen, 11720067)
10 ml	Hepes Buffer (+4 °C) Triiodothyronine
100 ml	Human Serum (thaw at 37 °C prior to use) (Gemini, 100-512)
20 ml	Antibiotic/Antimycotic (Invitrogen, 15240-062)
1 L	Basal Medium Eagle (Gibco, 21010046)

- Store at 4°C

Procedure

1. Start with 10,000-50,000 schistosomula in a single well of a six well plate, with 4 ml of medium. We use tissue-cultured treated plates. We do not add red blood cells at this stage.
2. Two days after the initial culture is set-up, tilt the 6 well plate at an angle and take out at least 3 ml of the "conditioned medium" being sure to leave the schistosomula behind. We syringe-filter the conditioned medium to avoid fungus contamination problems with the cultures.
3. Add 3 ml of SWAT and gently pipet the schistosomula in the SWAT using a P1000. Overly vigorous pipeting will cause schistosomula to float on the surface of the liquid.
4. Let the schistosomula settle (1-2 minutes) and remove as much of the added SWAT as possible, being sure to leave the schistosomula behind.
5. Add 3 ml of fresh medium plus 1 ml of conditioned medium.
6. Supplement with ~50 µl of packed mouse red blood cells. Red blood cells should be washed with SW once prior to use (this is done in a 15 ml conical tube, using approximately 1 ml of heparinized mouse blood plus 10 ml of SW, followed by centrifugation at 3000 rpm for 5 minutes).
7. Continue to feed and wash the schistosomula every other day (Monday, Wednesday, and Friday seems to work well). Supplement 3 ml of fresh medium with 1 ml of conditioned medium for as long as the conditioned medium lasts (~3-4 media changes). Once you run out of conditioned medium, add 4 ml of fresh medium and ~50 µl of packed mouse red blood cells to each well after you remove the SWAT from the wash.

Comments

Schistosomula can be cultured this way for at least two months. Approximately 50% of the schistosomula will not grow and very few (~10%) will grow to sexually distinct adults (mostly males). The biggest obstacle to success is fungal/bacterial contamination, probably a reflection of the fact that the parasites originate from non-sterile snails; this is the reason that we use more than the recommended concentration of Antibiotic/Antimycotic. This does not appear to affect parasite growth.

References

1. Basch PF. 1981. Cultivation of *Schistosoma mansoni in vitro*. I. Establishment of cultures from cercariae and development until pairing. Journal of Parasitology 67(2):179-85.
2. Tucker, M. S., Karunaratne, L. B., Lewis, F. A., Frietas, T. C., and Liang, Y-S. 2013. Schistosomiasis, in *Current Protocols in Immunology* 19.1.1-19.1.57, John Wiley and Sons, Inc., (R. Coico, Ed). Published online November 2013 in Wiley Online Library (wileyonlinelibrary.com). doi: 10.1002/0471142735.im1901s103.