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Establishment of primary intestinal epithelial cells and leukocytes from the three-spined stickleback, Gasterosteus aculeatus

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

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Abstract

This protocol details the Establishment of primary intestinal epithelial cells and leukocytes from the three-spined stickleback, Gasterosteus aculeatus.

Attachments

535-1117.docx

30KB

Materials

Stock solutions:

0.1M dithiothreitol:

	A	B
	DTT powder	155 mg
	dH2O DNase free	10 mL
	Aliquot in 1ml tubes	
	Store at -20 C	

Note

Highly unstable at RT.

Mucus removal solution:

	A	B
	1X HBSS	18.6 ml
	DTT solution	1 ml
	FBS	0.4 ml
	Divide into two 15 ml tubes	

Epithelial cells recovery solution:

	A	B
	EDTA	29.224 g
	FBS	0.4 ml
	HBSS	
	Adjust pH to 7.3 using hydrochl oric acid or sodium hydroxide.	

Enzymatic digestion solution:

	A	B
	LiberaseTM	1.4 Wünsch units/ml
	DNase I	24 U/ml
	1X HBSS	7 ml

Reagents:

- Dithiothreitol (DTT) **Thermo Fisher Scientific Catalog #**R0861
- Cell Dissociation Buffer enzyme-free Hanks Balanced Salt Solution **Thermo Fisher Scientific Catalog #**13150016
- Ethylenediaminetetraacetic acid 99% pure **Thermo Fisher Scientific Catalog #**AC118432500
- Thermo Scientific™ Deoxyribonuclease I bovine pancreas **Thermo Fisher Scientific Catalog #**AAJ62229MB
- Supply Solutions Liberase™ DL Research Grade low Dispase concentration 2x5mg **Fisher Scientific Catalog #**501003356
- Leibovitz's L-15 Medium **Thermo Fisher Catalog #**11415064
- Corning™ Penicillin-Streptomycin Solution **Fisher Scientific Catalog #**MT30002CI
- Corning™ Premium Fetal Bovine Serum **Fisher Scientific Catalog #**MT35015CV
- Leuko Spin Medium **pluriSelect Catalog #**SKU 60-00091-10

Equipment:

Equipment	
Centrifuge	NAME
Eppendorf	BRAND
5804	SKU

Equipment

Fisherbrand™ accumet™ AE150 Benchtop pH Meter	NAME
pH meter	TYPE
Fisherbrand™	BRAND
AE150	SKU
https://www.fishersci.com/shop/products/accumet-ae150-ph-benchtop-meter/13636AE153	LINK

Equipment

Cole-Parmer® INC-250 Series Mini CO2 Digital Incubator	NAME
Digital Incubator	TYPE
Cole Parmer	BRAND
71717	SKU
https://www.coleparmer.in/p/cole-parmer-inc-250-series-mini-co2-digital-incubator/71717	LINK

Equipment

Microscope: Leica DMi1	NAME
Microscope	TYPE
Leica	BRAND
DMi1	SKU
https://www.leica-microsystems.com/products/light-microscopes/p/leica-dmi1/	LINK

Stereoscope: Leica S7E

Equipment

Stereoscope	NAME
Stereoscope	TYPE
Leica	BRAND
S7E	SKU
https://www.leica-microsystems.com/products/?nlc=20211222-SFDC-013861&utm_source=google&utm_medium=cpc&utm_campaign=Microscope_General_Brand&utm_content=text_ad&utm_term=leica%20microscopes&gclid=CjwKCAjwm8WZBhBUEiwA178UnAglbfhHkpdQN-rVZaBuiRQJC1g5-0g9p6K	LINK

- Biosafety cabinet: Sterilgard III Advance

Before start

Fish dissection:

- Prepare one Petri dish On ice with 10 mL of 1X PBS for collecting the tissue.
- Prepare Two Petri dishes containing 10 mL of PBS 1X, 0.1% povidone-iodine and, two Petri dishes with 10 mL 1X PBS and place inside the biosafety cabinet to sterilize the intestine.

Fish dissection

1 After following approved euthanasia procedures, place the fish's body On ice .

2 Make a ventral incision from the cloaca to the jaw using sharp surgical scissors.

3 Make two lateral incisions just behind the opercular flaps down to the lateral line of the fish.

4 Using two pins, secure the fish on its dorsal side on a dissecting pad.

5 To detach the intestine, make a cut at pyloric caeca on one side and the cloaca on the other side.

6 Place intestine into a Petri dish containing 10 mL of cold 1X PBS.

7 Using forceps and mini dissecting scissors, open the intestine by making a longitudinal incision.

8 Bring the Petri dish containing the intestine into the biological safety cabinet and wash the intestine by submerging in two successive 0.1% povidone-iodine washes for 00:05:00 each.

5m

9 Wash twice for 00:05:00 each in a Petri dish containing 10 mL of cold 1x PBS to remove iodine.

5m

Mucus removal:

10 Transfer opened gut in 10 mL of mucus removal solution and incubate for 00:10:00 at 17 °C on a gyratory rocker for 00:10:00 .

20m

11 Resuspend the gut tissue in a fresh 10 mL of mucus removal solution and incubate again incubate for 00:10:00 at 17 °C on a gyratory rocker. 10m

Epithelial cells recovery and enzymatic digestion:

12 10m

Note

Enzymatic digestion is affected by the temperatures.

Transfer the gut tissue to 10 mL of epithelial cells recovery solution and incubate for 00:10:00 at 17 °C on a gyratory rocker.

13 In order to recover epithelial cells in the suspension, remove the gut tissue from Epithelial cells recovery solution and keep it On ice for enzymatic digestion step. Centrifuge the cell suspension at 300 x g for 00:10:00 at 17 °C. 10m

14 Remove the supernatant and resuspend the epithelial cell pellet in HBSS with 2% FBS and 1% Pen Strep.

15 Transfer the intestinal tissue from step 13 to 7 mL of enzymatic digestion solution, then incubate for 00:30:00 at 17 °C on a gyratory shaker. 30m

16 Collect and save the cell suspension at 17 °C.

17 Resuspend the remaining intestinal tissue removed from the enzymatic digestion solution in 7 mL of fresh enzymatic digestion solution for a second enzymatic digestion.

18 Incubate for an additional 00:30:00 at 17 °C on a gyratory shaker. 30m

19 Recover the cell suspension and pool with cell suspensions obtained from step 16 in a 15 ml conical tube and keep at 17 °C.

20 Filter the obtained cell suspension through a 40 µm mesh cell strainer into a new tube to remove cell clumps.

21 Centrifuge the obtained unicellular suspension at 300 x g for 00:10:00 at 17 °C . 10m

22 Resuspend the cell pellet in 5 mL L15 with 2% FBS and 1% pen Strep.

Density gradient:

23 Use double-density leukocyte isolation medium to recover all leukocytes from the cell suspension.

24 In a 15 mL conical tube, add 10 mL of the density medium.

25 Carefully layer 5 mL of the cell suspension onto the density medium and mix the two phases.

26 Centrifuge for 00:20:00 at 750 x g at 17 °C . 20m

27 After the density centrifugation, one white layer of cells appears between the L15 medium and Ficoll. Aspirate the top layer of the L15 medium.

28 Next, transfer the mononuclear and polymorphonuclear cell layer to a new conical tube, while making sure to not aspirate the Ficoll gradient with the cells. Wash the cells by centrifuging them at 17 °C , 300 x g , once with 10 mL of L15 2% FBS and 1% PenStrep.

Cell seeding:

29 Seed the cells into 96 wells plate at a density of $1x10^6$ cells/ml in L15 media with 10% FBS and 1% PenStrep.