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

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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

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PROTOCOL CITATION

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KEYWORDS

Three-spined stickleback, Primary cell culture, Epithelial cells, Leukocytes, Enzymatic digestion

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MATERIALS TEXT

**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 | |



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**Epithelial cells recovery solution:**

| A | B |
|---|---|
| EDTA | 29.224 g |
| FBS | 0.4 ml |
| HBSS | |
| Adjust pH to 7.3 using hydrochlo | |

**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 |



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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



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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 STARTING

**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.



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## 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.

5m

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.



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5m

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

## Mucus removal:

20m

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.

10m

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.

## Epithelial cells recovery and enzymatic digestion:

10m

12

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.

10m

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.

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



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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



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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.



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