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Processing of pediatric adenoid and tonsil samples for single cell analysis

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Liam Gubbels¹, Shivanthan Shanthikumar¹, Melanie R Neeland¹

¹Murdoch Children's Research Institute

earlyAIR



Liam Gubbels

Murdoch Children's Research Institute

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Protocol status: Working

We use this protocol and it's working

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Abstract

This protocol describes the collection, processing, and cryopreservation of pediatric adenoid and tonsil samples for downstream single-cell analysis.

Guidelines

This is an experimental protocol for the processing of adenoid and tonsil samples collected from children. Sample collection must have and be compliant with Human Ethics Committee approval.



Materials

- ✕ RPMI-1640 **Merck MilliporeSigma (Sigma-Aldrich) Catalog #R5886**
- ✕ Fetal Bovine Serum
- ✕ 1X PBS (Phosphate-buffered saline)
- ✕ DMSO (dimethyl sulfoxide) **Merck MilliporeSigma (Sigma-Aldrich) Catalog #D8418**
- ✕ conical tubes, 50ml
- ✕ conical tubes, 15ml
- ✕ 1.5 mL Eppendorf tubes
- ✕ Ficoll Paque PLUS **GE Healthcare Catalog #17144003-500 ml**
- ✕ Cell strainer, 100 μ m **VWR International (Avantor) Catalog #08-771- 19**
- ✕ FACS Tubes
- ✕ Acridine Orange/Propidium Iodide stain **Logos Biosystems Catalog #F23991**
- ✕ PhotonSlide **Logos Biosystems Catalog #L12007**
- ✕ Human TruStain FcX™ (Fc Receptor Blocking Solution) **BioLegend Catalog #422301, 422302**
- ✕ Petri Dish Glass 60mm x 15mm Borosilicate **Pacific Laboratory Products Catalog #LW2387-01**
- ✕ Forceps (tweezers), 12.5cm, Sharp End **Bio Basic Inc. Catalog #FC001.SIZE.1**
- ✕ Surgical scissors
- ✕ Blades Scalpel, Handle No. 4 **westlab Catalog #663-930**
- ✕ Scalpel Blades #22 **westlab Catalog #663-254**
- ✕ cryovial

Flow antibodies:  Flow cytometry panels.pdf 301.2KB

Equipment

Aurora - 5L Configuration

NAME

Spectral Flow Cytometer

TYPE

Cytek Aurora

BRAND

N/A

SKU

5L Configuration

SPECIFICATIONS



Equipment

Luna FL

NAME

Cell counter

TYPE

Luna

BRAND

L20001

SKU

<https://logosbio.com/luna-fl/>

LINK

Safety warnings



Human samples should be processed in a laboratory with appropriate biosafety infrastructure and procedures.



COLLECTION OF ADENOID AND TONSIL TISSUE

- 1 Prepare specimen containers for adenoid and tonsil samples by adding 10mL pre-chilled RPMI supplemented with 2% heat-inactivated fetal calf serum (referred to as RPMI 2% FCS).
- 2 After obtaining informed consent from family and/or patient, collect adenoids/tonsils at the time of clinically indicated tonsillectomy/adenoidectomy.

Note

For guidelines on how to safely collect tonsil/adenoid tissue from children, please see:

Citation

Mitchell RB, Archer SM, Ishman SL, Rosenfeld RM, Coles S, Finestone SA, Friedman NR, Giordano T, Hildrew DM, Kim TW, Lloyd RM, Parikh SR, Shulman ST, Walner DL, Walsh SA, Nnacheta LC (1970) . Clinical Practice Guideline: Tonsillectomy in Children (Update)-Executive Summary.

<https://doi.org/10.1177/0194599818807917>

LINK

- 3 Adenoid and tonsil samples must be placed on ice and processed in the laboratory within  00:30:00 to  01:00:00 of the procedure.

PROCESSING OF ADENOID AND TONSIL TISSUE TO CELL SUSPENSION

1h 50m

- 4 Place the tissue in a glass cell culture plate with 10mL RPMI 2% FCS. Remove any visible blood clots, fat, and connective tissues with forceps and scissors/scalpel 10m
- 5 Transfer the trimmed adenoid/tonsil tissue to a new glass cell culture plate containing 10mL RPMI 2% FCS. Mince the tissue into a fine paste using scissors or a scalpel. 10m
- 6 Muddle the tissue using a plunger from a sterile syringe to dissociate the cells from the tissue, then filter the cell suspension through a 100µm cell strainer into a 50mL tube. 15m



Centrifuge the cell suspension  400 x g, 4°C, 00:05:00

Note

Cell isolation can also be done using a gentleMACS tissue dissociator

- 7 Remove the supernatant and resuspend the cell pellet in 8mL RPMI 2% FCS. Fill a 15mL tube with 2mL of Ficoll plaque plus and layer the adenoid/tonsil cell suspension onto the surface of the Ficoll solution. 5m

Note

Layer the cell suspension slowly to prevent the Ficoll solution from mixing with the cells.

- 8 Centrifuge the layered cell suspension at 45m

 400 x g, Room temperature, 00:30:00 , 4 Acceleration and NO brake .

- 9 Once the spin is complete, carefully aspirate the mononuclear layer at the interface between the RPMI 2% FCS and the Ficoll solution into a new 15mL tube. Top up the cell suspension to 10mL with RPMI 2% FCS and centrifuge  400 x g, 4°C, 00:05:00 5m

Note

When collecting the cells, try to avoid Ficoll solution as much as possible.

- 10 Discard supernatant and resuspend cell pellet in 3 mL RPMI 2% FCS. 5m

- 11 Prepare cell suspension for cell counting. Here, we use AO/PI and the LUNA FL counter. Dilute cell suspension in a microcentrifuge tube in RPMI 2% FCS at a ratio of 1:10 for adenoids and 1:100 for tonsils. Remove 18µL of diluted cells and place into a new microcentrifuge tube for cell counting. Add 2 µL of AO/PI to the count tube and mix well. 5m

- 11.1 Load 10µL of stained cells onto a LUNA fluorescent counting slide and count. Record viability, total cell count, and live cell count.

**Note**

Cell counting can also be performed manually using a haemocytometer, or using other automated cell counters.

12 If choosing to run flow cytometry or other single cell assays on fresh cells, here is where you can allocate the required number of cells for downstream processing. For flow cytometry, described below, we allocate 500,000 cells.

13 Top up the cell suspension to 10mL with RPMI 2% FCS and centrifuge

 400 x g, 4°C, 00:05:00 .

5m

CRYOPRESERVATION OF ADENOID AND TONSIL MONONUCLEAR CELLS

10m

14 Discard supernatant and resuspend cells at a ratio of 1:1 in RPMI 2% FCS and freeze solution (heat-inactivated FCS + 15% DMSO) such that cells are frozen between 1-20 million cells/mL. Transfer cells to cryogenic vial.

10m

15 Immediately place cryogenic vials into an isopropanol freezing container (e.g. Nalgene® Mr. Frosty) or Cool Cell (Corning) and transfer to  -80 °C overnight.

16 For long term storage, transfer the vials to liquid nitrogen.

PREPARATION OF CELLS FOR FLOW CYTOMETRY

5m

17 Resuspend cell suspension for fixable viability staining according to manufacturers' instructions (e.g. the LIVE/DEAD™ Fixable UV Blue Stain from Invitrogen/ThermoFisher).

18 Following the required incubation, stop the reaction by the addition of 1mL staining buffer (2% heat-inactivated FCS in PBMS 2 mM EDTA, herein referred to as FACS buffer) and centrifuge at

 400 x g, 4°C, 00:05:00

5m

19 Resuspend cells in 25µL FACS buffer and add 15µL FC-block for  00:05:00 at

 Room temperature

5m

20 The next steps will depend on the requirements for your specific panel. As an example, we have attached our 31-plex spectral cytometry panel that we routinely use on cells isolated from tonsil and adenoid tissue. All of the following steps are related to this panel.

 tonsil_adenoid_blood_panel.pdf 127.6KB

- 21 Add 10 μ L of Brilliant Stain Buffer (Becton Dickinson) and then add 25 μ L of Cocktail 1A made up at 3X concentration and incubate for

10m

 00:10:00 In the dark at room temperature

- 22 Then, directly add cockta made up at 2X concentration 1:1 with cells and incubate for

30m

 00:30:00 In the dark at room temperature

- 23 Following staining, wash cells with 2mL FACS buffer and centrifuge at

5m

 400 x g, 4°C, 00:05:00 and resuspend cells in 100 μ L FACS buffer for acquisition on a flow cytometer (here, a Cytex 5L aurora).

Note

Panels 1A and 1B were adapted from: "OMIP-069: Forty-Color Full Spectrum Flow Cytometry Panel for Deep Immunophenotyping of Major Cell Subsets in Human Peripheral Blood" and we thank the authors for their detailed methods.

Citation

Park LM, Lannigan J, Jaimes MC (1970)
. OMIP-069: Forty-Color Full Spectrum Flow Cytometry Panel for Deep
Immunophenotyping of Major Cell Subsets in Human Peripheral Blood.

<https://doi.org/10.1002/cyto.a.24213>

[LINK](#)