May 08, 2024

Processing of pediatric adenoid and tonsil samples for single cell analysis

DOI
dx.doi.org/10.17504/protocols.io.3byl49ewjgo5/v1

Liam Gubbels[1], Shivanthan Shanthikumar[1], Melanie R Neeland[1]

[1]Murdoch Children's Research Institute

earlyAIR

Liam Gubbels
Murdoch Children's Research Institute

OPEN ACCESS

DOI: dx.doi.org/10.17504/protocols.io.3byl49ewjgo5/v1

Protocol Citation: Liam Gubbels, Shivanthan Shanthikumar, Melanie R Neeland 2024. Processing of pediatric adenoid and tonsil samples for single cell analysis. **protocols.io** https://dx.doi.org/10.17504/protocols.io.3byl49ewjgo5/v1

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

Created: March 20, 2024

Last Modified: May 08, 2024

Protocol Integer ID: 96965

Keywords: single-cell, flow cytometry, respiratory, Paediatric, tonsils, adenoids

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 **Sigma Aldrich Catalog #**R5886
- Fetal Bovine Serum
- 1X PBS (Phosphate-buffered saline)
- DMSO (dimethyl sulfoxide) **Sigma Aldrich Catalog #**D8418
- conical tubes, 50ml
- conical tubes, 15ml
- 1.5 mL Eppendorf tubes **Contributed by users**
- Ficoll Paque PLUS **GE Healthcare Catalog #**17144003-500 ml
- Cell strainer, 100 µm **VWR International Catalog #**08-771- 19
- FACS Tubes **Contributed by users**
- 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 **Contributed by users**
- Blades Scalpel, Handle No. 4 **westlab Catalog #**663-930
- Scalpel Blades #22 **westlab Catalog #**663-254
- cryovial **Contributed by users**

Flow antibodies: Flow cytometry panels.pdf 301KB

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 (2019). Clinical Practice Guideline: Tonsillectomy in Children (Update)-Executive Summary..

LINK

https://doi.org/10.1177/0194599818807917

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. Centrifuge the cell suspension 400 x g, 4°C, 00:05:00 15m

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 400 x g, Room temperature, 00:30:00 , 4 Acceleration and NO brake . 45m

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 127KB

21 Add 10µL of Brilliant Stain Buffer (Becton Dickinson) and then add 25µL of Cocktail 1A made up at 3X concentration and incubate for 00:10:00 In the dark at room temperature 10m

22 Then, directly add cockta made up at 2X concentration 1:1 with cells and incubate for 00:30:00 In the dark at room temperature 30m

23 Following staining, wash cells with 2mL FACS buffer and centrifuge at 400 x g, 4°C, 00:05:00 and resuspend cells in 100µL FACS buffer for acquisition on a flow cytometer (here, a Cytek 5L aurora). 5m

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 (2020). OMIP-069: Forty-Color Full Spectrum Flow Cytometry Panel for Deep Immunophenotyping of Major Cell Subsets in Human Peripheral Blood..
LINK
https://doi.org/10.1002/cyto.a.24213