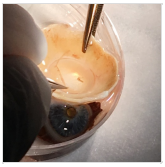

May 06, 2020

Single-Cell Dissociation of Human Trabecular Meshwork

Alexi Mcadams[1], Tavé van Zyl[1]

[1]Department of Ophthalmology, Harvard Medical School; Glaucoma Service, Mass. Eye and Ear, Boston, MA

1 Works for me dx.doi.org/10.17504/protocols.io.bfdyji7w

Human Cell Atlas Method Development Community

Alexi Mcadams

ABSTRACT

Fresh trabecular meshwork tissue is dissected from post-mortem human eyes and dissociated into a single cell suspension.

THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

van Zyl T, Yan W, McAdams A, Peng YR, Shekhar K, Regev A, Juric D, Sanes JR. Cell atlas of aqueous humor outflow pathways in eyes of humans and four model species provides insight into glaucoma pathogenesis. Proceedings of the National Academy of Sciences. 2020 Apr 27.

GUIDELINES

The stock solutions can be prepared up to 6 months in advance and aliquots can be stored at -20˚C.

MATERIALS

NAME	CATALOG #	VENDOR
Sodium bicarbonate	S6014	Sigma Aldrich
RNase Zap	R2020-250ML	Sigma Aldrich
Razor blade		
Papain	LS003126	Worthington Biochemical Corporation
Ames' Medium	A1420	Sigma Aldrich
Bovine Serum Albumin (Non-acetylated)	B6917	Sigma Aldrich
Bovine Serum Albumin	A9418	Sigma Aldrich
Deoxyribonuclease I from bovine pancreas	D4527	Sigma Aldrich
Trypsin Inhibitor Ovomucoid	LS003087	Worthington Biochemical Corporation
L-Cysteine hydrochloride	C1276	Sigma Aldrich
40 um Falcon® Cell Strainers Sterile Corning	21008-949	VWR international Ltd
Millex-GP Syringe Filter Unit 0.22 µm polyethersulfone 33 mm gamma sterilized	SLGP033RS	Emd Millipore
70% EtOH		
Disposable Petri Dishes		
Blunt curved Westcott scissors		
Vannas Microscissors		
Jewellers Forceps		
50 mL Falcon Tubes		
15 mL Falcon Tubes		
1.7 mL Eppendorf Tubes		

STEPS MATERIALS

Citation: Alexi Mcadams, TavÃÂ© van Zyl (05/06/2020). Single-Cell Dissociation of Human Trabecular Meshwork . https://dx.doi.org/10.17504/protocols.io.bfdyji7w

NAME	CATALOG #	VENDOR
L-Cysteine hydrochloride	C1276	Sigma Aldrich
Sodium bicarbonate	S6014	Sigma Aldrich
Ames' Medium	A1420	Sigma Aldrich
Deoxyribonuclease I from bovine pancreas	D4527	Sigma Aldrich
Bovine Serum Albumin (Non-acetylated)	B6917	Sigma Aldrich
Bovine Serum Albumin	A9418	Sigma Aldrich
Trypsin Inhibitor Ovomucoid	LS003087	Worthington Biochemical Corporation
Papain	LS003126	Worthington Biochemical Corporation
RNaseZap™ RNase Decontamination Solution	AM9780	Thermo Fisher Scientific

SAFETY WARNINGS

Universal biosafety precautions should be observed with the handling of all human post-mortem tissue. Instruments should be cleaned and sterilized appropriately after each dissection.

BEFORE STARTING

Fresh Ames' medium should be prepared and oxygenated at the beginning of each dissection to allow for fresh working solutions.

Prepare Stock Solutions 2h

1 Prepare Oxygenated Ames' Medium

- Add 1.9 g sodium bicarbonate to a 1L container (e.g. glass bottle or flask)
- Empty one container of Ames' Medium powder to 1L bottle
- Fill bottle with 1L of ddH_2O, mix well
- Bubble oxygen gas (95% O2/5% CO2) through solution

Sodium bicarbonate
by Sigma Aldrich
Catalog #: S6014

Ames' Medium
by Sigma Aldrich
Catalog #: A1420

2 DNase stock solution

- DNase I 40,000 Units
- oxygenated Ames' solution 3 mL

Filter solution into new 15 mL falcon tube with 0.22 um filter to sterilize

- Store 115 µL aliquots at -20˚C.

Citation: Alexi Mcadams, TavÃÂ© van Zyl (05/06/2020). Single-Cell Dissociation of Human Trabecular Meshwork . https://dx.doi.org/10.17504/protocols.io.bfdyji7w

Deoxyribonuclease I from bovine pancreas
by Sigma Aldrich
Catalog #: D4527

3 **0.4% non-acetylated BSA stock solution**

- NA BSA 25 mg
- oxygenated Ames' solution 6.25 mL

Filter solution into new 15 mL falcon tube with 0.22 um filter to sterilize

- Store 100 µL aliquots at -20˚C.

Bovine Serum Albumin (Non-acetylated)
by Sigma Aldrich
Catalog #: B6917

4 **Prepare 10X Ovomucoid solution (LO)**

- BSA 150 mg
- Ovomucoid 150 mg
- Oxygenated Ames' 10 mL
- dissolve and adjust pH to ~7.4 with a few µL of NaOH (2N)

Filter solution into new falcon tube with 0.22 um filter to sterilize

- Make 1 mL aliquots & store at -20˚C.

Bovine Serum Albumin
by Sigma Aldrich
Catalog #: A9418

Trypsin Inhibitor Ovomucoid
by Worthington Biochemical Corporation
Catalog #: LS003087

5 **L-cysteine stock solution**

- L-cysteine, anhydrous (sigma C1276): 24 mg
- ddH2O 1 mL

Citation: Alexi Mcadams, TavÃÂ© van Zyl (05/06/2020). Single-Cell Dissociation of Human Trabecular Meshwork . https://dx.doi.org/10.17504/protocols.io.bfdyji7w

Filter into new 15 mL falcon tube with 0.22 um filter to sterilize

- Store 115 µL aliquots at -20˚C.

L-Cysteine hydrochloride
by Sigma Aldrich
Catalog #: C1276

Prepare Working Solutions and Materials 1h

6 Prepare Fresh Oxygenated Ames' Medium

- Add 1.9 g sodium bicarbonate to a 1L container (e.g. glass bottle or flask)
- Empty one container of Ames' Medium powder to 1L container
- Fill container with 1L of ddH_2O, shake to mix
- Bubble oxygen gas (95% O2/5% CO2) through solution

Sodium bicarbonate
by Sigma Aldrich
Catalog #: S6014

Ames' Medium
by Sigma Aldrich
Catalog #: A1420

7 Prepare papain solution

- Oxygenated Ames' 10 mL
- Papain 200 Units (ex: for a 44.8 mgP/mL, 26.6 u/mgP batch, use 168 uL)
- DNase I stock solution 100 uL

Filter into new 15 mL falcon tube with 0.22 um filter to sterilize

- L-cysteine aliquot (see above, stock solution) 100 uL

Activate the papain by adding the L-cysteine and incubating at 37 ˚C for 15 minutes before using for dissociation.

Papain
by Worthington Biochemical Corporation
Catalog #: LS003126

Citation: Alexi Mcadams, TavÃÂ© van Zyl (05/06/2020). Single-Cell Dissociation of Human Trabecular Meshwork . https://dx.doi.org/10.17504/protocols.io.bfdyji7w

8 **Prepare 1X Ovomucoid solution (LO)**

- 10X LO (aliquot) 1 mL
- Oxygenated Ames' 9 mL

9 **Prepare 0.04% non-acetylated BSA**

- Oxygenated Ames' 900 uL
- 0.4% non-acetylated BSA stock 100 uL

Tissue collection & dissection 45m

10 **Set up dissection area**

- Clean all intruments and workspace with RNaseZap then 70% EtOH.
- Place all instruments on a Chux Pad or similar sheet to work on

Note: Keep the eye in immersed in Ames media or Optisol within a container on ice until dissection; and preferrably, the eye should be processed within 6 hours from death.

RNaseZap™ RNase Decontamination Solution
by Thermo Fisher Scientific
Catalog #: AM9780

11 **Start Dissection under microscope: Isolate the anterior segment**

- Make a small incision at the pars plana (~4 mm posterior to the limbus) with a razor blade.
- Cut circumferentially with curved scissors to liberate the anterior portion from the rest of the eye.
- Place the anterior segment in oxygenated Ames' medium.

12 **Expose the trabecular meshwork**

- Free the lens from the ciliary body by carefully cutting the zonules with curved microscissors all the way around the lens
- Gently lift the lens from the rest of the structures with the scissor blades (holding the scissors parallel with the workbench)
- Take care and try to avoid violating or puncturing the lens capsule
- Gently peel the ciliary body and iris from the corneoscleral button with forceps.
- Trim any large chunks of ciliary muscle left behind from the trabecular meshwork with microscissors.
- This is an ideal time to begin activating the papain solution in a 37˚C waterbath

13 **Isolate the trabecular meshwork**

- Identify the TM and insert one prong of the Jewelers forceps into Schlemm's canal
- Grab the TM with the forceps and gently pull the TM out in strips. Some of the ciliary muscle and possibly descemet membrane will come along, and this is okay for the purposes of this protocol
- The back wall of schlemm canal can also be gently scraped so that cells here can be transferred to the papain solution

Citation: Alexi Mcadams, TavÃÂ© van Zyl (05/06/2020). Single-Cell Dissociation of Human Trabecular Meshwork . https://dx.doi.org/10.17504/protocols.io.bfdyji7w

Dissociate tissue into single cell solution 1h

14 **Digest tissue**

- Add TM strips to prewarmed, **activated papain solution** in an eppendorf tube
- Incubate for a total of 30 minutes at 37 ˚C
- Every 7-10 minutes, invert tube to agitate gently

15 **Dissociate tissue into a single cell suspension**

- When finished incubating, centrifuge digested tissue and papain solution for 30 seconds at 500 rcf
- Carefully remove supernatant to leave behind tissue
- Add 1 mL **1X LO** to the tissue
- Triturate with a 1000 uL pipette
- Filter through a 40 um strainer into a 50 mL falcon tube
- If any large chunks of tissue are left behind, return to eppendorf and add another 1 mL of **1X LO** and repeat.
- Rinse filter with 500 uL - 1000 uL of **1X LO** and collect in the same 50 mL falcon tube

16 **Prepare single cell solution for loading onto 10X**

- Place the 50ml falcon tube containing the cell/1X LO suspension in centrifuge
- Centrifuge cells suspension at 500 rcf for 5 minutes (preferably at 4 ˚C)
- Carefully remove supernatant. Note where pellet should be given the falcon tubes' position in the centrifuge; sometimes the pellet is too small to see
- Resuspend the cells in 50 uL - 200 uL **0.04% non-acetylated BSA**, depending on pellet size
- Transfer to a 1.7 mL eppendorf tube
- Count cells with hemocytometer and dilute with **0.04% non-acetylated BSA** to desired concentration based on 10X protocol (we usually aim to achieve a concentration of 1000 cells per microliter and load the 10X for 6000 cells recovered)
- Cell viability assay can be performed at this stage as well prior to loading

Citation: Alexi Mcadams, TavÃÂ© van Zyl (05/06/2020). Single-Cell Dissociation of Human Trabecular Meshwork . https://dx.doi.org/10.17504/protocols.io.bfdyji7w