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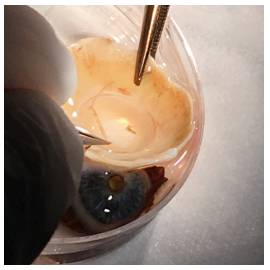
Single-Cell Dissociation of Human Trabecular Meshwork



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Human Cell Atlas Metho...

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We use this protocol and it's working

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Abstract

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

Guidelines

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

Materials

MATERIALS

- Sodium bicarbonate **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**S6014
- RNase Zap **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**R2020-250ML
- Razor blade
- Papain **Worthington Biochemical Corporation** **Catalog #**LS003126
- Ames' Medium **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**A1420
- Bovine Serum Albumin (Non-acetylated) **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**B6917
- Bovine Serum Albumin **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**A9418
- Deoxyribonuclease I from bovine pancreas **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**D4527
- Trypsin Inhibitor Ovomucoid **Worthington Biochemical Corporation** **Catalog #**LS003087
- L-Cysteine hydrochloride **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**C1276
- 40 um Falcon® Cell Strainers Sterile Corning **VWR International (Avantor)** **Catalog #**21008-949
- Millex-GP Syringe Filter Unit 0.22 µm polyethersulfone 33 mm gamma sterilized **Merck Millipore (EMD Millipore)** **Catalog #**SLGP033RS
- 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

STEP MATERIALS

- L-Cysteine hydrochloride **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**C1276
- Sodium bicarbonate **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**S6014
- Ames' Medium **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**A1420
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Sodium bicarbonate **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**S6014

Ames' Medium **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**A1420

Papain **Worthington Biochemical Corporation** **Catalog #**LS003126

RNaseZap™ RNase Decontamination Solution **Thermo Fisher Scientific** **Catalog #**AM9780

Protocol materials

Sodium bicarbonate **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**S6014

Trypsin Inhibitor Ovomucoid **Worthington Biochemical Corporation** **Catalog #**LS003087

Ames' Medium **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**A1420

Deoxyribonuclease I from bovine pancreas **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**D4527

Disposable Petri Dishes

Jewellers Forceps

15 mL Falcon Tubes

Razor blade

RNase Zap **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**R2020-250ML

Millex-GP Syringe Filter Unit 0.22 µm polyethersulfone 33 mm gamma sterilized **Merck Millipore (EMD Millipore)** **Catalog #**SLGP033RS

Papain **Worthington Biochemical Corporation** **Catalog #**LS003126

L-Cysteine hydrochloride **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**C1276

50 mL Falcon Tubes

Bovine Serum Albumin **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**A9418

RNaseZap™ RNase Decontamination Solution **Thermo Fisher Scientific** **Catalog #**AM9780

40 um Falcon® Cell Strainers Sterile Corning **VWR International (Avantor)** **Catalog #**21008-949

Bovine Serum Albumin (Non-acetylated) **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**B6917

70% EtOH

Blunt curved Westcott scissors

Vannas Microscissors

1.7 mL Eppendorf Tubes

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 start

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 **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**S6014

Ames' Medium **Merck MilliporeSigma (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.

Deoxyribonuclease I from bovine pancreas **Merck MilliporeSigma (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) **Merck MilliporeSigma (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 **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**A9418

Trypsin Inhibitor Ovomucoid **Worthington Biochemical Corporation Catalog #**LS003087

5 **L-cysteine stock solution**

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

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

- Store 115 µL aliquots at -20˚C.

L-Cysteine hydrochloride **Merck MilliporeSigma (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 **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**S6014

Ames' Medium **Merck MilliporeSigma (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 **Worthington Biochemical Corporation Catalog #**LS003126

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 **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

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