Isolation of Myoepithelial Cells from Adult Murine Lacrimal and Submandibular Glands

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Abstract

The lacrimal gland (LG) is an exocrine tubuloacinar gland that secretes an aqueous layer of tear film. The LG epithelial tree is comprised of acinar, ductal epithelial, and myoepithelial cells (MECs). MECs express alpha smooth muscle actin (α SMA) and have a contractile function. They are found in multiple glandular organs and are of ectodermal origin. In addition, the LG contains SMA+ vascular smooth muscle cells of endodermal origin called pericytes: contractile cells that envelop the surface of vascular tubes. A new protocol allows us to isolate both MECs and pericytes from adult murine LGs and submandibular glands (SMGs). The protocol is based on the genetic labeling of MECs and pericytes using the *SMA^{CreErt2/+};Rosa26-TdTomato^{fl/fl}* mouse strain, followed by preparation of the LG single-cell suspension for fluorescence activated cell sorting (FACS). The protocol allows for the separation of these two cell populations of different origins based on the expression of the epithelial cell adhesion molecule (EpCAM) by MECs, whereas pericytes do not express EpCAM. Isolated cells could be used for cell cultivation or gene expression analysis.

SUMMARY:

The lacrimal gland (LG) has two cell types expressing α -smooth muscle actin (α SMA): myoepithelial cells (MECs) and pericytes. MECs are of ectodermal origin, found in many glandular tissues, while pericytes are vascular smooth muscle cells of endodermal origin. This protocol isolates MECs and pericytes from murine LGs.

Keywords

Myoepithelial cells; lacrimal gland; smooth muscle actin; epithelial; single-cell isolation

INTRODUCTION:

Myoepithelial cells (MECs) are present in many exocrine glands including lacrimal, salivary, harderian, sweat, prostate, and mammary. MECs are a unique cell type that combines an epithelial and a smooth muscle phenotype. MECs express α -smooth muscle actin (SMA) and have a contractile function^{1,2}. In addition to MECs, the lacrimal gland (LG) and the

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submandibular gland (SMG) contains SMA+ vascular cells called pericytes, which are cells of endodermal origin that envelop the surface of vascular tubes³. Although MECs and pericytes express many markers, SMA is the only marker that is not expressed in other LG and SMG cells^{1,3}.

Within the last 40 years, several laboratories reported assays for dissociation of different exocrine gland tissues, in which non-enzymatic and enzymatic approaches were applied. In one of the first reports published in 1980, Fritz and coauthors described a protocol to isolate feline parotid acini using sequential digestion in a collagenase/trypsin solution⁴. In 1989, Hann and coauthors adjusted this protocol for acini isolation from rat LGs using a mixture of collagenase, hyaluronidase and DNase⁵. In 1990, Cripps and colleagues published the method of non-enzymatic dissociation of lacrimal gland acini⁶. Later, in 1998, Zoukhri and coauthors returned to an enzymatic dissociation protocol for following up Ca²⁺-imaging on LG and SMG isolated acini⁷. Within the last decade, researchers have turned their focus on isolation of stem/progenitor cells from exocrine glands. Pringle and coauthors described a protocol in 2011 for isolation of mouse SMG stem cells⁸. This method was based on isolation of stem cell-containing salispheres, which were maintained in culture. The authors claimed that proliferating cells expressing stem cell-associated markers could be isolated from these salispheres⁸. Shatos and coauthors published the protocol for progenitor cell isolation from uninjured adult rat LGs using enzymatic digestion and collecting “liberated” cells⁹. Later, in 2015, Ackermann and coauthors adjusted this procedure to isolate presumptive “murine lacrimal gland stem cells” (“mLGSCs”) that could be propagated as a mono-layer culture over multiple passages¹⁰. However, none of the before mentioned procedures allowed for distinguishing cellular subtypes and individual populations of isolated epithelial cells. In 2016, Gromova and coauthors published a procedure for isolation of LG stem/progenitor cells from adult murine LGs using FACS¹¹. However, this protocol was not intended to isolate MECs.

Recently, we have shown that we are able to isolate SMA+ cells from 3-week-old SMA-GFP mice¹². However, at this time we have not separated different populations of SMA+ cells. Here we established a new procedure for the direct isolation of differentiated MECs and pericytes from adult LGs and SMGs.

PROTOCOL:

All animal work was conducted according to the National Institute of Health (NIH) guidelines and was approved by Institutional Animal Care and Use Committee of the Scripps Research Institute. All efforts were made to minimize the number of mice and their suffering. All experimental animals received a standard diet with free access to tap water.

NOTE: The main steps for MEC and pericyte isolation are outlined schematically in Figure 1A–F. All reagents and equipment used for this procedure are described in Table 1.

1. Mice and labelling the SMA cells

- 1.1. Use adult (2–4 months old) tamoxifen-inducible, α SMA driven reporter mice *SMA^{CreErt2/+};Rosa26-TdTomato^{fl/fl}*.

NOTE: The *SMA^{CreErt2}* strain was kindly provided by Dr. Ivo Kalajzic¹³. *Rosa26-TdTomato^{fl/fl}* (*B6.Cg-Gt(ROSA)26Sor^{tm9(CAG-tdTomato)Hze/J}*, also known as *Ai9*) strain (#007909) were purchased from Jackson Laboratory (Sacramento, CA). SMA+ cells were labeled by intraperitoneal tamoxifen (TM) administration.

1.2. Preparation of tamoxifen solution

1.2.1. Prepare filtered corn oil. Use 0.22 µm vacuum filter since corn oil is viscous.

1.2.2. Transfer 1 g of TM powder from the bottle into a 50 mL tube. Add 1 mL of ethanol to the bottle, cap and shake it to rinse then add to a 50 mL tube. Repeat once more with another 1 mL of ethanol.

1.2.3. Add filtered corn oil to make 50 mL of a 20 mg/mL TM solution. Vortex the tube, wrap it in foil, and put it in a shaking water bath or shaking incubator at 45 °C.

1.2.4. It may take about 12–24 h to dissolve the TM. From time to time, remove the tube and check for any remaining crystals. Once the TM is completely dissolved, aliquot and store at –80 °C. A thawed aliquot can be reused.

1.3. To label SMA+ cells, inject mice intraperitoneally (IP) with TM on two sequential days.

1.3.1. Inject 3–4 weeks old *SMA^{CreErt2/+};*Rosa26-TdTomato^{fl/f}** any gender mice with TM at 100 µL/20 g (or 2 mg/20 g) body weight (Figure 1A). Mice are ready to be used for cell isolation in 2–3 days after the last TM injection. If needed, injected mice can be sacrificed at longer periods of time after TM injection.

NOTE: As controls for proper compensation during FACS, one wild type (C57Bl/6) mouse and one *SMA^{CreErt2/+};*Rosa26-TdTomato^{fl/fl}** mouse not injected with TM (with “unstained” MECs) of the same age would be required. Use the same calculations provided for 2 *SMA^{CreErt2/+};*Rosa26-TdTomato^{fl/fl}** mice. Not injected *SMA^{CreErt2/+};*Rosa26-TdTomato^{fl/fl}** will allow evaluation of the DSRed background. The C57Bl/6 mouse will serve as a negative control of unstained cells.

2. Solutions and buffers

NOTE: The LG is an epithelial origin gland that contains an extracellular matrix that makes dissociation of cells difficult. Therefore, using a special combination of enzymes and a multistep digestion process described below is recommended.

2.1. Dispase type II stock solution (25x)

2.1.1. Dissolve 120 mg of dispase type II powder in 2 mL of 50 mM HEPES/150 mM NaCl to prepare a 25x stock solution (final concentration of dispase should be 30 Units/mL). Units per milligram may vary depending on the number of mice and concentration of the dispase should be adjusted accordingly.

2.1.2. Prepare 200 µL aliquots and store them at –70 °C for up to 6 months or 4 °C for several days. Do not freeze/thaw the aliquot of dispase more than once to prevent enzyme degradation.

2.2. DNase type I stock solution

- 2.2.1.** Dissolve 5 mg of DNase type I powder in a 5 mL solution of 50% glycerol, 20 mM Tris buffer (pH 7.5), and 1 mM MgCl₂ (stock concentration should be approximately 2000 Units/mL). Units per milligram may vary depending on number of mice and thus concentration of DNase should be adjusted accordingly.
- 2.2.2.** Filter the stock solution using a 0.22 µm filter and a 10 mL syringe.
- 2.2.3.** Prepare 200 µL aliquots and store them at –70 °C for up to 6 months or 4 °C for several days. Do not freeze/thaw more than once to prevent enzyme degradation.

2.3. Digestion medium

- 2.3.1.** To 10 mL of DMEM low glucose without glutamine, add 100 µL of cell culture supplement (e.g., Glutamax, see Table of Materials) for a dilution of 1:100.
- 2.3.2.** To 2 mL of DMEM low glucose with cell culture supplement, add 6 mg of Collagenase type I and mix thoroughly by pipetting (enzyme on wet ice), 160 µL of dispase stock solution (2.4 U/mL final concentration), 16 µL of DNase type I stock solution (8 U/mL final concentration), and 12 µL of 1 M CaCl₂ (6 mM final concentration).

NOTE: Calcium is required to increase enzymatic activity^{14,15}. All calculations are provided for isolation of cells from four lacrimal glands from two adult mice. The volume of digestion medium may vary depending on the amount of tissue and number of replicates. Do not use more than 4 lacrimal glands from 2–4 months old mice per 2 mL medium.

2.4. Blocking medium I

- 2.4.1.** To 25 mL of DMEM/F-12, add FBS (15% final concentration), 250 µL of cell culture supplement (see Table of Materials) for a dilution of 1:100, and 50 µL of 0.5 M EDTA pH 8.0 (1 mM final concentration).

NOTE: Of the different types of medium that were compared for this protocol DMEM/F-12 gave the best results. This medium has also been used by other researchers to isolate/culture epithelial cells^{16,17}.

2.5. Blocking medium II

- 2.5.1.** To 25 mL of PBS, add 50 µL of 0.5 M EDTA pH 8.0 (1 mM final concentration).

2.6. Recovery medium

- 2.6.1.** To 2 mL of HBSS supplemented with 5 mM MgCl₂, add 100 µL of DNase type I stock solution to 100 U per 2 mL final concentration. Relatively high concentrations of DNase-type I is required to reduce aggregation of epithelial cells.

2.7. Fluorescence activated cell sorting (FACS) buffer

- 2.7.1.** To 486.5 mL of PBS, add 12.5 mL of serum (2.5% final concentration) and

1 mL of 0.5 M EDTA pH 8.0 (1 mM final concentration).

NOTE: The buffer can be stored at 4 °C for a maximum of 6 weeks.

3. Adult mouse lacrimal gland harvesting and microdissection

- 3.1. Anesthetize the mouse by isoflurane inhalation (adjust the isoflurane flow rate or concentration to 5% or greater) and sacrifice by cervical dislocation. Perform anesthesia and euthanasia according to institutional IACUCs recommendations.
- 3.2. Using fine forceps and scissors, remove skin between the eye and ear (Figure 2A).
- 3.3. To dissect a LG, gently pull LG using tweezers and at the same time scratch the connective tissue around the LG using the sharp tip of small scissors to free it (Figure 2B).
- 3.4. Avoid cutting with scissors, as the LG and parotid salivary glands are located very close to each other and must be separated prior to dissection. When LG and parotid glands are separated, cut the LG out using scissors. Place glands into a 35 mm dish with 2 mL of cold PBS (keep on ice) (Figure 1B).
- 3.5. As the LG is covered by a connective tissue capsule/envelope, trim any surrounding fat and connective tissue under a dissecting microscope and remove the LG capsule with two forceps.
 - 3.5.1. Repeat this step for all glands.
- 3.6. Check a small piece of tissue under fluorescent microscope to ensure cell labeling (Figure 1C).

4. Preparation of LG single-cell suspension

- 4.1. Transfer all LGs into a 35 mm dish with 0.5 mL of room temperature (RT) digestion media and mince LGs using small scissors into very small pieces (approximately $0.2\text{--}1\ \mu\text{m}^2$). Normally, it takes about 3 min to mince 4 LGs (Figure 2C).
- 4.2. Transfer minced tissue into a 2 mL round bottom tube using a wide-bore pipette filter tip. Use a normal sized pipette tip with the tip cut off (Figure 2D).
- 4.3. Add up to 2 mL digestion medium and mix by inverting the tube.
- 4.4. Place tube in a shaking incubator (or shaking water bath), at 37 °C, 100–120 rpm for 90 min.
- 4.5. Every 30 min slowly pipette gland pieces 20–30 times using a 1,000 μL filter tip with the decreasing bore size (Figure 2D). After incubation/trituration, take a 10 μL aliquot and inspect under a microscope for clusters. If clusters persist, continue digestion.
- 4.6. After 90 min, pass the sample 2–3 times through an insulin syringe needle (31G) to further release cells into suspension.

NOTE: No visible lacrimal gland pieces should remain in the solution once digestion is completed (Figure 1D).

- 4.7. Transfer cell suspension to a 15 mL tube and add blocking media type I to a total of 5 mL. Invert the tube 2–3 times to mix.
- 4.8. Pass the cell suspension through a 70 μ m cell strainer placed on a 50 mL tube. Wash the strainer with 1 mL of blocking media type I. Repeat step 4.8 again.
- 4.9. Centrifuge samples at 1,242 x *g* for 5 min at RT.
- 4.10. Aspirate the supernatant. Re-suspend cells in 2 mL of blocking medium type II using a 1 mL pipette tip and transfer the cell suspension into a 2 mL microcentrifuge tube.
- 4.11. Centrifuge the cells at 0.4 x *g* (24 $\times$ 1.5/2.0 mL rotor; see Table of Materials) for 3 min at RT.
- 4.12. Aspirate supernatant and re-suspend cells in 1 mL of cell detachment solution (see Table of Materials).

NOTE: Here, the cell detachment solution is Accutase, a marine-origin enzyme with proteolytic and collagenolytic activity that detaches/dissociates cells for analysis of cell surface markers.

- 4.13. Incubate cells at 37 °C, at 100–120 rpm for 2–3 min. Over-digestion with cell detachment solution may damage cellular membranes.
- 4.14. Transfer cell suspension into a 50 mL tube and add up 10 mL of blocking medium type I. Centrifuge tube at 0.4 x *g* (24 $\times$ 1.5/2.0 mL rotor; see Table of Materials) for 5 min.
- 4.15. Discard supernatant and re-suspend cells in 6 mL of recovery media and incubate cells for 30 min at RT.
- 4.16. Check 10 μ L of cell suspension under the microscope to ensure complete cell dissociation (Figure 4).
- 4.17. Count cells using a cell counter and Trypan blue. Normally, we expect 4 $\times$ 10⁵-6 $\times$ 10⁶ cells from four LGs (one sample).
- 4.18. Centrifuge cells at 0.4 x *g* (24 $\times$ 1.5/2.0 mL rotor; see Table of Materials) for 3 min at RT and proceed to antibody staining.

5. Antibody staining

- 5.1. Add up to 5 $\times$ 10⁵ cells to a 2 ml tube containing 400 μ L of FACS buffer. Add 5 μ L of Brilliant Violet 421 anti-mouse CD326 (EpCAM) and 0.5 μ L of Ghost Red 780 (Viability Dye).
- 5.2. In parallel, prepare controls to adjust FACS compensation:

Negative control-1 (cells from wild type mouse)

Background control unstained cells from *SMA^{CreErt2/+};*Rosa26-TdTomato^{fl/fl}**

Cy7–780 stained cells (cells from the wild type mouse stained with the Ghost Red 780 Viability Dye)

EpCAM-Brilliant Violet 421 (cells from wild type mouse stained with the EpCAM-Brilliant Violet 421 antibody).

NOTE: For each control sample use a minimum of 1×10^5 cells per 400 μ L of FACS buffer.

- 5.3. After adding each reagent mix cells thoroughly by pipetting.
- 5.4. Wrap tube(s) with foil and rotate tubes for 45 min at 4 °C.
- 5.5. Centrifuge samples at $0.4 \times g$ ($24 \times 1.5/2.0$ mL rotor; see Table of Materials) for 3 min at 4°C.
- 5.6. Re-suspend cells in 1 mL of FACS buffer. It is important to wash cells to decrease the background during compensation.

6. Fluorescence activated cell sorting

- 6.1. Transfer cell suspension into 5 mL FACS tubes and proceed with FACS analysis. Keep cells on ice.
- 6.2. Adjust compensation using single color controls.
- 6.3. Sort cells at 20 psi through a 100 μ m nozzle using appropriate flow cytometer (see Table of Materials). Gating strategy¹⁸ is shown in Figure 1E and Figure 3.
- 6.4. Collect sorted cells into medium, RNA-later, FACS or lysis buffers depending on downstream procedures (Figure 1E,F).

REPRESENTATIVE RESULTS:

Mouse model to isolate SMA+ MECs and pericytes

The established protocol allows for the isolation of two pure populations: MECs and pericytes from LGs and SMGs (see Table 1). These two types of cells have a different size and appearance. Microvascular pericytes, develop around the walls of capillaries (Figure 5A) and have a squared shape (Figure 5B), while MECs surround the LG secretory acini, have long processes and occupy a relatively large area (Figure 5A,B). The described procedure is based on genetic cell labeling of SMA+ in the TM-inducible *SMA^{CreErt2/+};Rosa26-TdTomato^{fl/fl}* mouse strain. Additionally, EpCAM antibodies allow the researchers to distinguish epithelial SMA⁺:EpCAM⁺ cells of ectodermal origin (MECs) and SMA⁺:EpCAM⁻ cells of endodermal origin (pericytes).

Preparation of single-cell suspension

The LG contains a filamentous extracellular matrix that must be digested thoroughly. The provided protocol allows preparation of a single cell solution for FACS analysis and further applications. The example of dissociated cells is shown in Figure 4.

MEC and pericyte isolation by FACS

To distinguish MECs from pericytes, single cells were stained with antibody to EpCAM, which detects only epithelial cells. The main population of cells was determined by forward and side scatter area gating (Figure 3A). Doublets were excluded by plotting the forward scatter area versus width and with the side scatter area versus width (Figure 3B). Dead cell exclusion was done via Ghost Red 780 (Viability Dye) (Figure 3C). An unlabeled control (Figure 3E), background control and antibody control labeled with a single primary antibody were used to determine the background noise (nonspecific antibody binding) and to establish proper compensation for optimum separation between signals (Figure 3D). Data analyses were performed by using FlowJo software.

MECs and pericytes were gated by DsRed labeling. DsRed⁺ dim (not shown) and DsRed⁺ bright cells within both MEC and pericyte cell populations were detected (Figure 4D). The brightness of labeled cells may depend on level of SMA expression or degree of reporter activation upon TM injection¹⁹. Only DS Red⁺ bright cell population were collected since only fully differentiated cells were required. The DS Red⁺ dim cell populations require further investigation.

Downstream applications

It is well known that MECs play an important contractile function in exocrine glands. Moreover, they are very plastic cells and have features of stem cells. Therefore, isolated MECs could be used in multiple applications. For example, cells can be cultured, used for RNA isolation or transplantation (Figure 1F)^{12,20–22}.

DISCUSSION:

This manuscript described a protocol of MEC and pericyte isolation from LG and SMG. This procedure was based on genetic labeling of SMA, the only reliable biomarker of MECs and pericytes.

The urgency to develop this protocol was motivated by the almost total absence of literature highlighting the isolation of MECs from murine LGs and SMGs. Although genetic labeling was previously used, using SMA-GFP mice to isolate SMA⁺ cells from young three-week-old LGs¹², it did not allow the use these older mice for cell isolation due to partial loss of signal in adult mice. In addition, GFP-labeled cells give a relatively high background in FACS applications²³ and require additional compensations. In contrast, the *SMA^{CreErt2/+}:Rosa26-TdTomato^{fl/fl}* mouse line shows no or a low background and high levels of SMA labeling activation throughout mouse postnatal development, adulthood and aging. Usage of the *SMA^{CreErt2/+}:Rosa26-TdTomato^{fl/fl}* mouse is especially important for studies focused on disease progression or aging, since SMA⁺ cells in these mice could be labeled prior to disease development and studied later when disease/aging progresses. Another critical step of the protocol is thorough LG mincing and the following digestion. This step may reduce the cell number obtained during FACS analysis. Overall, isolation and immediate analysis of primary cells is also important due to significant changes in the transcriptional profiles of cells maintained in culture²⁴.

Additionally, the described protocol for MEC and pericyte isolation from murine LGs enables different downstream procedures. Protein, RNA and DNA extractions are possible from both single-cell populations, although several mice/samples need to be processed in parallel or sequentially to increase the number of cells. Taken together, the obtained results demonstrate an effective and relatively straightforward way for MEC and pericyte isolation from different murine glandular tissue.

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Figure 1. A schematic representation of the experiment.

(A) IP injections of the *SMA^{CreErt2/+};Rosa26-TdTomato^{fl/fl}* mice with TM. (B) Isolation and mincing of the LG or SMG. (C) Analysis of cell labeling using fluorescence microscope. (D) Multi-step enzymatic digestion to prepare a single-cell solution. It is critical to check digestion steps under a light microscope to ensure that cells are released from clusters. (E) Example of gating showing SMA+ bright DS Red+/EpCAM+ (MECs) and DS Red+bright/EpCAM-(pericytes). (F) Collected MECs and pericytes could be subjected to different downstream procedures including cell cultivation, RNA isolation and gene expression analysis and cell transplantation.

Figure 2. Critical steps of LG isolation/mincing.

(A) Removal of skin between the eye and ear to dissect LG. Dashed yellow circle indicates LG location. (B) LG dissection. Yellow arrowhead points out area between lacrimal and parotid glands. (C) LG mincing in digestion medium using scissors with curved, blunt ends. (D) Transfer of minced tissue into 2 mL tube. Yellow arrowhead depicts wide bore size 1 ml tip required for transferring.

Figure 3. Identification of murine LG MECs and pericytes using FACS.

(A) Determination of the main population of LG cells by forward and side scatter area gating. (B) Exclusion of doublets via forward scatter area versus width. (C) Dead cell exclusion via Ghost Red 780 (Viability Dye). (D) MEC (SMA^+ bright/ EpCAM^+) and pericyte (SMA^+ bright/ EpCAM^-) populations distinguished based on staining with EpCAM antibody. (E) Unlabeled control (cells from wild type mouse). On each plot the % of gated cells is provided.

Figure 4. Confocal and differential interference contrast (DIC) images illustrating dissociated single cells from murine LG.

(A-C) single cells isolated from two LGs of one 4 month old *SMA^{CreErt2/+};Rosa26-TdTomato^{fl/fl}* mouse. Nuclei are stained with DAPI (blue). White arrowheads denote SMA+ (DS Red) cells: MECs or pericytes. Scale bar is 15 μ m.

Figure 5. Confocal images showing difference in distribution and shape between MECs and pericytes isolated from murine LG.

(A) Whole mount preparation of LG with labeled cells showing difference in distribution between MEC and pericytes. (B) The shape of MEC and pericyte is different. Pericyte is relatively small and has squarish shape, whereas MEC is large and has irregular shape and several long processes. Arrowheads: MECs and pericytes.

Table 1.
Modifications of the protocol for isolation of cells from the submandibular gland (SMG).

The table describes major modifications required to isolate MECs and pericytes from murine SMG in comparison with the procedure for murine LG.

Name	Company	Catalog Number	Comments
Biosafety Cabinet	SterilCard Baker	19669.1	Class II type A/B3
10 mL Disposable serological pipets	VWR	89130–910	Manufactured from polystyrene and are supplied sterile and plugged
10 mL Disposable serological pipets	VWR	89130–908	Manufactured from polystyrene and are supplied sterile and plugged
15 mL High-clarity polypropylene conical tubes	Falcon	352196	
25 mL Disposable serological pipets	VWR	89130–900	Manufactured from polystyrene and are supplied sterile and plugged
5 mL FACS round-bottom tubes	Fisher Scientific, Falcon	14–959-11A	
50 mL High-clarity polypropylene conical tubes	Falcon	352070	
Antibiotic-antimycotic	Invitrogen	15240–062	
Appropriate filter and non-filter tips	Any available	Any available	
BD Insulin Syringes	Becton Dickinson	328468	with BD Ultra-Fine needle 1/2 mL 8 mm 31 G
BD Syringes 10 mL	Becton Dickinson	309604	Sterile
Brilliant Violet 421 anti mouse CD326 (EpCAM)	Biolegend	118225	Monoclonal Antibody (G8.8)
CaCl ₂ 1M solution	BioVision	B1010	sterile
Cell culture dishes 35 mm	Corning	430165	Non-pyrogenic, sterile
Collagenase Type I	Worthington	LS004194	
Corn oil	Any available	Any available	From grocery store
Corning cell strainer size 70 µm	Sigma-Aldrich	CLS431751–50EA	
Digital Stirrer PC-410D	Corning	Item# UX-84302–50	
Dispase II	Sigma-Aldrich	D4693–1G	
Dissecting scissors, curved blunt	McKesson Argent	487350	Metzenbaum 5–1/2 Inch surgical grade stainless steel non-sterile finger ring handle
DNase I	Akron Biotech, catalog number	AK37778–0050	
Dulbecco's Modified Eagle's Medium – low glucose (DMEM)	Sigma-Aldrich	D5546–500ML	with 1,000 mg/L glucose and sodium bicarbonate, without L-glutamine
Dulbecco's Modified Eagle's Medium/F12 (DMEM/F12)	Millipore	DF-042-B	without HEPES, L-glutamine
Easypet 3 pipette controller	Eppendorf	4430000018	with 2 membrane filters 0.45 µm, 0.1 – 100 mL
Ethanol	Sigma-Aldrich	E7023–500ML	
Ethylenediaminetetraacetic acid (EDTA)	Sigma-Aldrich	E6758	
Fisher Vortex Genie 2	Fisher Scientific	12–812	

Name	Company	Catalog Number	Comments
FlowJo version 10	Any available	Any available	
Fluorescence binocular microscope Axioplan2	Carl Zeiss	ID# 094207	
Ghost Red 780 Viability Dye	Tonbo Biosciences	13-0865-T100	
GlutaMAX Supplement	ThermoFisher Scientific, Gibco	35050061	
Glycerol 99%	Sigma-Aldrich	G-5516	
Hand tally counter	Heathrow Scientific	HEA6594	
Hank's Balanced Salt Solution (HBSS)	Sigma Millipore	H6648-500ML	Modified, with sodium bicarbonate, without calcium chloride, magnesium sulphate, phenol red.
Hank's Balanced Salt Solution (HBSS)	ThermoFisher Scientific	14025092	With calcium, magnesium, no phenol red.
Hausser Bright-Line Phase Hemocytometer	Fisher Scientific	02-671-51B	02-671-51B
HEPES 1 M solution	ThermoFisher Scientific, Gibco	15630-080	Dilute 1/10 in ddH2O
HyClone Fetal Bovine Serum (FBS)	Fisher Scientific	SH3007002E	
Hydrochloric Acid (HCl), 5 N Volumetric Solution	JT Baker	5618-03	To adjust Tris buffer pH
Innova 4230 Refrigerated Benchtop Incubator	New Brunswick Scientific	SKU#:	Shaker; 37 °C, 5% CO ₂ in air
Iris scissors	Aurora Surgical	AS12-021	Pointed tips, delicate, curved, 9 cm, ring handle
Isoflurane Inhalation Anesthetic	Southern Anesthesia Surgical (SAS)	PIR001325-EA	
MgCl ₂ 1 M solution	Sigma-Aldrich	63069-100ML	
Microcentrifuge tubes 1.5 mL	ThermoFisher Scientific	3451	Clear, graduated, sterile
Microsoft Power Point	Any available	Any available	
NaCl powder	Sigma-Aldrich	S-3014	
Nalgene 25 mm Syringe Filters	Fisher Scientific	724-2020	
Pen Strep	Gibco	15140-122	
pH 510 series Benchtop Meter	Oakton	SKU: BZA630092	
Phosphate buffered saline (PBS)	ThermoFisher Scientific	10010023	pH 7.4
Pure Ethanol 200 Proof	Pharmco-Aaper	111000200	
Red blood cell lysis buffer 10x	BioVision	5831-100	
Roto-torque Heavy Duty Rotator	Cole Parmer	MPN: 7637-01	
Safe-lock round bottom Eppendorf tubes 2 mL	Eppendorf Biopur	22600044	PCR inhibitor, pyrogen and RNase-free
Scissors	Office Depot	375667	
Sorting flow cytometer MoFlo Astrios EQ	Beckman Coulter	B25982	With Summit 6.3 software
Sorvall Legend Micro 17R Microcentrifuge	Thermo Scientific	75002441	All centrifugation performed at RT
Sorvall RT7 Plus Benchtop Refrigerated Centrifuge	Thermo Scientific	ID# 21550	RTH-750 Rotor. All centrifugation performed at RT
Stemi SV6 stereo dissecting microscope	Carl Zeiss	455054SV6	With transmitted light base
Tamoxifen	Millipore Sigma	T5648-1G	

Name	Company	Catalog Number	Comments
Trizma base powder	Sigma-Aldrich	T1503	
Trypan blue solution	Millipore Sigma	T8154	
Two Dumont tweezers #5	World Precision Instruments	500342	11 cm, Straight, 0.1 mm x 0.06 mm tips
Upright microscope	Any available	Any available	With transmitted light base
Vacuum filtration systems, standard line	VWR	10040–436	
Variable volume micropipettes	Any available	Any available	