



## A simple and effective method to consistently isolate mouse cardiomyocytes

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### Abstract

The need for reproducible yet technically simple methods yielding high-quality cardiomyocytes is essential for research in cardiac biology. Performing cellular and molecular functional experiments (e.g., contraction, electrophysiology, calcium cycling, etc.) on cardiomyocytes are the gold standard for establishing mechanism(s) of disease. Mouse is the species of choice for functional experiments and the described technique is specifically for the isolation of mouse cardiomyocytes. Previous methods requiring a Langendorff apparatus require high levels of training and precision for aortic cannulation, often resulting in ischemia. The field is shifting towards Langendorff-free isolation methods which are simple, reproducible, and yield viable myocytes for physiological data acquisition and culture. These methods greatly diminish ischemia time compared to aortic cannulation and result in reliably obtained cardiomyocytes. Our adaptation to the Langendorff free method includes an initial perfusion with ice-cold clearing solution, use of stabilizing platform that ensures a steady needle during perfusion, and additional digestion steps to ensure reliably obtained cardiomyocytes for use in functional measurements and culture. This method is simple and quick to perform and requires little technical skill.

### SUMMARY:

The gold standard in cardiology for cellular and molecular functional experiments are cardiomyocytes. This article describes adaptations to the non-Langendorff technique to isolate mouse cardiomyocytes.

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#### DISCLOSURES:

No conflicts of interest to disclose.

## INTRODUCTION:

For decades, an essential idea in cardiac biology literature is molecular mechanism of action. Mechanism of action must be established in order to publish reliable studies. A well-established strategy to determine molecular mechanism is isolated cardiomyocyte studies, which require high-quality cardiomyocytes for attaining trustable data. Cellular and molecular experiments performed on cardiomyocytes to determine mechanism of action are the gold standard for investigating contraction<sup>1</sup>, electrophysiology<sup>2</sup>, calcium (Ca<sup>2+</sup>) cycling<sup>3</sup>, myofilament Ca<sup>2+</sup> sensitivity<sup>4</sup>, cytoskeletal<sup>5</sup>, metabolism<sup>6</sup>, effects of hormones<sup>7</sup>, signaling molecules<sup>8</sup>, drug studies<sup>9</sup>, etc. Mouse has become the species of choice for most cardiac biology experiments due to the ease of genetic manipulation, small size, relatively short lifespan, cost, etc<sup>10</sup>. However, the reliable isolation of high-quality mouse cardiomyocytes is not trivial with current techniques.

Labs have been isolating cardiomyocytes for almost 70 years<sup>11</sup>. Virtually all techniques to isolate cardiomyocytes rely on digestion of the heart via various enzymes (collagenase, protease, trypsin, etc). In the early periods (1950s-60s), the chunk method was employed, which involves removing the heart, cutting into much smaller pieces and incubating in solution with collagenase/protease/trypsin<sup>12</sup>. With updates to the “Langendorff apparatus”<sup>13</sup>, in the 1970s labs began isolating cardiomyocytes using the coronary artery perfusion-based isolation technique (retrograde perfusion with enzyme via the Langendorff apparatus), and remains the dominant method of myocyte isolation in the field today, ~50 years later<sup>14–16</sup>. Recent work has shifted to cannulating the heart *in vivo* to limit hypoxia time and ischemic damage resulting in superior cardiomyocyte isolations (better yields and higher quality).<sup>17</sup> Recently, this has evolved into performing *in vivo*, Langendorff-free heart perfusions<sup>18–22</sup>. We have evolved the Langendorff-free cardiomyocyte isolation technique based on the Ackers-Johnson et al.<sup>18</sup> technique and adapted various components from the many previous isolation techniques. These key adaptations include injection of an ice-cold clearing buffer, the incorporation of a supporting platform to stabilize the needle, allowing for decreased manipulation of the heart. Also detailed in this technique is a temperature control of injected buffers via a warming jacket (37°C), decreased time between *in vivo* injection and digestion due to less EDTA perfusion as previously published<sup>18</sup>. By decreasing manipulation of the heart and therefore minimizing puncture site size, thorough and constant perfusion of the coronary arteries is obtained. We also added additional digestions by using the chunk isolation method, decreased the amount of EDTA in the injected clearing buffer, and changed the pH. Our described technique is more reliable, more efficient, and does not require the extensive training/practice compared to the using the Langendorff apparatus (see Table 1).

## PROTOCOL:

All procedures performed in this study were approved by the Institutional Animal Care and Use Committee at the Ohio State University in accordance with NIH guidelines.

### 1. SOLUTION PREPARATION

#### 1.1 Before Isolation (up to 2 weeks in advance of myocyte isolation)

- 1.1.1** Perfusion Buffer Base: Prepare 1 L of perfusion buffer base (NaCl, KCl, NaH<sub>2</sub>PO<sub>4</sub>, HEPES, Glucose, BDM) in 1 L of ultrapure 18.2 MΩ cm H<sub>2</sub>O. Adjust to pH 7.4 prior to 0.22 μm sterile filtration. Store at 4 °C until day of isolation.
- 1.1.2** Clearing buffer: Prepare 1 L of Clearing buffer (NaCl, KCl, NaH<sub>2</sub>PO<sub>4</sub>, HEPES, Glucose, EDTA, BDM) in 1 L of ultrapure 18.2 MΩ cm H<sub>2</sub>O. Adjust to pH 7.4 prior to 0.22 μm sterile filtration. Store at 4 °C until day of isolation.
- 1.1.3** 100 mM CaCl<sub>2</sub> Stock Solution: Weigh out 1.11 g of CaCl<sub>2</sub> and dissolve into 100 mL of ultrapure 18.2 MΩ cm H<sub>2</sub>O. Store at room temperature until day of isolation.
- 1.1.4** Liberase Aliquots: Dissolve 5 mg of digestive enzymes in 5 mL of sterile, RNase-free water. Prepare 0.8 mL aliquots and store at -20 °C until day of isolation.
- 1.1.5** Laminate Dishes: Apply 100 μL of 40 μL/mL mouse laminin to sterile, glass bottom 30 mL petri dishes. Allow to sit for 30 min and remove excess laminin. Set laminin on petri dish with UV incubation at room temperature for 30 min. Store at 4 °C until day of isolation (laminated petri dishes can be used up to 2 weeks).
- 1.1.6** DMEM Media: In a biosafety cabinet, add 2.5 mL FBS, 0.5 mL Penicillin-Streptomycin (50 U/mL – 50 μg), 1mL 500 mM BDM to 45.5 mL DMEM. Store at 4 °C until day of isolation (media can be used up to 2 weeks).
- 1.1.7** M199 Media: Prepare 1 L of M199 Media (NaHCO<sub>3</sub>, HEPES, L-glutathione, M199, BDM, BSA) in 1 L of ultrapure 18.2 MΩ cm H<sub>2</sub>O. Adjust to pH 7.4 prior to sterile filtration (0.22 μm filter). Store at 4 °C until day of isolation.
- 1.1.8** 500 mM BDM Stock: Add 0.5 g BDM to 10 mL ultrapure 18.2 MΩ cm H<sub>2</sub>O. Store at 4 °C until day of isolation.
- 1.1.9** Stabilizing platform creation: Mold play dough around the base of the needle screwed into the luer stopcock. Mold to the petri dish that will contain the heart and ensure that the needle is at proper height for ventricle injection (~5 mm above bottom of dish).

## 1.2 The Day of Isolation

- 1.2.1** Perfusion Buffer: The day of the isolation, add 9.5 mg of MgCl<sub>2</sub> to 100 mL perfusion buffer base. Allow to mix completely before removing 30 mL of completed perfusion buffer and place in a clean, wide-mouth 100 mL glass bottle with a screw cap lid (Digestion Buffer). These

additions can be made the day of isolation without additional pH adjustments as their addition negligibly alters the pH. Remove 12 mL of perfusion buffer and set to the side (Stop Buffer). Pour the remaining 58 mL of perfusion buffer into another clean, wide-mouth 100 mL glass bottle with a screw cap lid. Store the remaining perfusion buffer at 37 °C throughout isolation.

**1.2.2** Digestion Buffer: Add 7.5  $\mu$ L of 100 mM CaCl<sub>2</sub> to 30 mL of perfusion buffer for a final concentration of 25  $\mu$ M. Immediately prior to the isolation, add 770  $\mu$ L of Liberase TH to the digestion buffer for a final Liberase concentration of 26  $\mu$ g/mL.

**1.2.3** Stop Buffer: Dissolve 240 mg of BSA in 12 mL of perfusion buffer. Store at 37 °C throughout isolation.

**1.2.4** Clearing Buffer: Prepare a 3 mL syringe of Clearing Buffer and clear the needle of bubbles. Store on ice until isolation.

## 2. MANIFOLD PREPARATION

**2.1** Clear the temperature-controlled manifold with freshly prepared perfusion buffer, careful to ensure no bubbles remain. Using a luer lock connector, screw in a 26 G needle and clear of bubbles. A cleared manifold is essential for a successful isolation.

## 3. ANIMAL PREPARATION

**3.1** Inject the mouse with 100 mg/kg ketamine and 20 mg/kg xylazine by body weight immediately prior to the isolation. This procedure used a male, 4-month-old C57Bl/6 mouse.

**3.2** It may be helpful to place the mouse on a heated surgical pad to encourage sedation. Tent the arms of the mouse and tape the base of the tail to a blue lab diaper. Ensure that the animal is fully sedated by toe-pinch reflex.

## 4. CARDIOMYOCYTE ISOLATION PROCEDURE

**4.1** Expose the sternum of the mouse and, lateral to the midline, cut proximally through the ribs and to the axilla. Gently cut fully through the diaphragm, ensuring shallow cuts to avoid the heart. Clamp the sternum with a hemostat and fold the ribs backwards to expose the thoracic cavity.

**4.2** Gently remove the pericardium from the heart and cut fully through the inferior vena cava, immediately distal to the heart. Use a 3mL syringe with a 26G needle to quickly inject 3 mL ice-cold Clearing Buffer into the right ventricle of the heart over 1 minute

- 4.3 Gently hold the heart using tweezers and pull away from the body, exposing as much of the aorta as possible. Using a hemostat, clamp the ascending aorta, being careful not to clamp the atria, and excise the heart from the chest.
- 4.4 Quickly transfer the clamped heart to the lid of a polypropylene petri dish with approximately 10 mL of warm perfusion buffer. Place the clamped heart into the supporting platform and use a stabilized 26G needle attached to the manifold to inject 10 mL of temperature-controlled 37 °C perfusion buffer into the left ventricle over 5 minutes.
- 4.5 Transfer the clamped heart and supporting platform to a petri dish with approximately 5 mL of digestion buffer. Exchange input syringes for a 50 mL syringe with 25 mL digestion buffer.
- 4.6 Clear the manifold of any bubbles and remaining perfusion buffer before injection. Carefully replace needle into same needle position in left ventricular apex.
- 4.7 Use a perfusion pump to inject temperature-controlled 37 °C digestion buffer into the left ventricle for 15 minutes using a 50 mL syringe. The solution should be temperature-controlled by a water jacket previously calibrated to eject 37 °C solution at the end of the needle.
- 4.8 Remove the ventricles from the atria and transfer to a 10 mL beaker. Add 3 mL digestion buffer to the beaker and, using sharp scissors, cut the ventricles into large chunks. Cover the beaker with aluminum foil and place in a shaking water bath preheated to 37 °C for 5 minutes.
- 4.9 Discard the supernatant, careful to avoid removing any chunks of tissue. Resuspend the tissue chunks in 3 mL digestion buffer and triturate for approximately 4 minutes or until a homogenous mixture is achieved.
- 4.10 Filter cells through 70 um nylon cell strainer into a 50 mL polypropylene tube.
- 4.11 Return filtrate to a 14 mL round-bottom polypropylene falcon tube and centrifuge for 1 minute at 100 rpm. Discard the supernatant and resuspend the pellet in 3 mL of stop buffer.
- 4.12 Add 54 µL of 100 mM CaCl<sub>2</sub> stock to the resuspended cells for a final CaCl<sub>2</sub> concentration of 1.8 mM. This step can also be performed stepwise to increase cell yield.
- 4.13 Allow live cells to gravity settle for 10 minutes before removing supernatant containing dead cells. Resuspend pellet in storage solution (perfusion solution with 200 µM Ca). These cells can now be used for functional experiments (i.e., Ca Imaging/contraction, patch clamp, etc.), culture, etc.

**4.14** Cells may also be resuspended in lab and experiment dependent solutions.

## 5. CELL CULTURE

- 5.1** Count cells using a hemocytometer or by field view count using a gridded cover slip. As myocytes are very large, counting using a hemocytometer is not always accurate. This is used to get an idea of approximately how many cells were obtained from the isolation.
- 5.2** Dilute cells to ~25,000 cells/mL with DMEM Media. Add 1 mL of cell solution to each well.
- 5.3** Incubate at 37 °C, 95% O<sub>2</sub> 5% CO<sub>2</sub> for 2 hrs.
- 5.4** Gently aspirate DMEM media from each well and add 2 mL of M199 media.
- 5.5** Incubate at 37 °C, 95% O<sub>2</sub> 5% CO<sub>2</sub> for up to 24 hrs.

## REPRESENTATIVE RESULTS:

There are a few elements to examine when determining the success of an isolation. First, the cardiomyocytes must be rod-shaped with no membrane blebs, such as the cells isolated in figures 1A and 1B. A typical isolation will yield ~80% of the myocytes being rod-shaped. If the isolation yields anything less than 50% rod shaped cells, then it is considered an unsuccessful isolation and cardiomyocytes are not used. Lastly, the cardiomyocytes should be quiescent. The myocytes spontaneously contracting demonstrates that they are not Ca<sup>2+</sup> tolerant and will produce unreliable data. Of all the isolations performed using the above technique, nearly all isolations are considered successful. Cultured cardiomyocytes are considered successful if they are rod-shaped with no membrane blebs or rounded edges with high survival rates (figure 2).

## DISCUSSION:

The principal advantage of our Langendorff-free cardiomyocyte isolation technique is that it limits hypoxia and ischemic time by not requiring cannulation to a Langendorff apparatus. Alternatively to classical Langendorff techniques which require minutes to remove, clean, and hang the heart, often resulting in ischemic damage to the myocyte, our method includes an in vivo clearing of blood via ice-cold clearing solution. The ice-cold clearing buffer contains ethylenediaminetetraacetic acid (EDTA), which irreversibly chelates divalent cations that efficiently removes calcium, making it an excellent anticoagulant, and stops contraction<sup>23</sup>. Using ice-cold solutions will inhibit contraction, which will slow the metabolic rate preventing damage caused to ischemia<sup>24</sup>. By removing the need for aortic cannulation, Langendorff-free techniques produces robust myocytes resulting in a more reliable preparation to yield trustworthy data, which is not always the case for Langendorff methods of isolation.

This is a variation of a previously published Langendorff-free isolation technique<sup>18</sup> with adaptations as highlighted in Table 1. Most importantly, this technique introduces a stabilizing platform which limits the number of times the needle must be removed and replaced from the heart. Any unnecessary movement introduced to the needle while inserted into the ventricle widens the puncture site. A wider puncture site results in backflow out of the ventricle from the puncture site, decreased pressure in the heart, and decreased flow to the coronaries. This issue is fixed by using the stabilizing platform. By limiting the needle movement and the number of times the needle is removed and replaced (one compared to three times as previously described<sup>18</sup>), we maintain consistent flow to the coronaries and consistently achieve digestion. Another key adaptation was the addition of a temperature controlled jacket to maintain enzyme solutions at 37°C upon entering the heart (temperature bath was calibrated to eject at 37°C from the needle). The digestion enzyme used has optimum reaction activity at 37°C, and the addition of temperature control increases enzyme activity, therefore decreasing digestion time. Liberase also has minimal lot-to-lot variability and has higher reproducibility compared to other enzymes. Another adaptation of previous techniques is decreased amount of injected clearing solution. Decreasing the amount of clearing buffer to enter the heart and allowing for more blood to be cleared by perfusion buffer decreases inactivation of the digestion enzyme by EDTA. Another reason that our method consistently achieves successful myocyte isolations is by usage of BDM. BDM is a myosin inhibitor and inhibits the power stroke mechanism of the sarcomere, thereby inhibiting contraction and limiting reoxygenation injury during digestion. By limiting contraction, we prevent calcium cycling and inherent reactive oxygen species production in contracting myocytes in culture. Since our culture media contains calcium, it is possible that the myocytes could contract and decrease subsequent yield after culture. We elect to culture our myocytes in BDM to prevent contraction and increase yield<sup>25,26</sup>. BDM can always be washed out of myocytes for functional measurements after culture with great success. Alternatively, all steps of this procedure can be performed without the addition of BDM, but isolations may not be considered as “successful” on BDM-free isolated myocytes.

Besides ischemic time, there are many other factors that will determine if an isolation will produce robust myocytes. Since there are different conditions in the individual labs, the persons performing the isolation may need to modify the amount of enzyme and/or calcium, the perfusion time and/or pump speed, and the rocking water bath speed and/or time. These parameters will be dependent upon the mouse model used, such as healthy or diseased (e.g., aging, etc.).

While this technique does not require surgical skill needed for the Langendorff based techniques, there are still critical steps to make the technique successful. The most common issue that yields poor myocytes with this technique is due to bubbles entering the perfusion system and blocking the myocardial tissue from access to perfusing buffer. The solution to this problem is careful practice to avoid bubbles (via proper syringe clearance technique, proper needle clearance technique, burping the manifold of bubbles when changing syringes, etc), as well as multiple exit points from the perfusion system in case a bubble is lodged in the manifold. Without bubbles, in our experience, we get near 100% successful myocyte isolations (defined as above 50% rod shaped myocytes, average is ~80%).



While the method has been simplified by removal of the surgical skill required for the Langendorff method, this adapted method has only been tried in mouse. Another advantage of the Langendorff-free method is that it can be used for many murine models (i.e., from neonate to senescence), as previously described.<sup>19</sup> Unfortunately, larger mammals (i.e., dog, pig, etc.) will not be suitable for this technique due to the size of their hearts. It would be impossible to perfuse the heart with enough solution to acquire reliable myocytes.

Our adaption of the Langendorff-free method is a dependable method for successful isolation of mouse cardiomyocytes. Compared to the Langendorff method, this alternative approach requires little technical skill to consistently obtain cardiomyocytes.

## ACKNOWLEDGMENTS:

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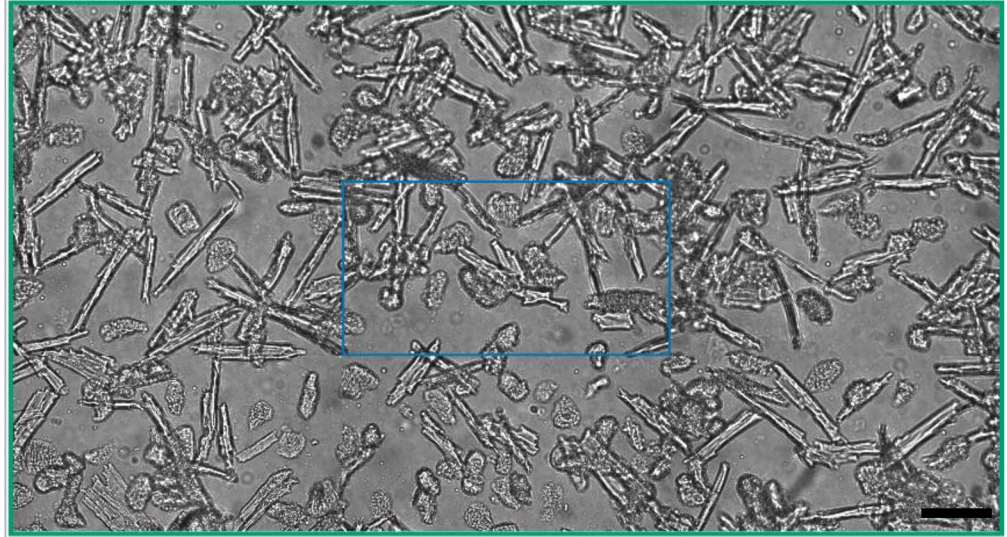
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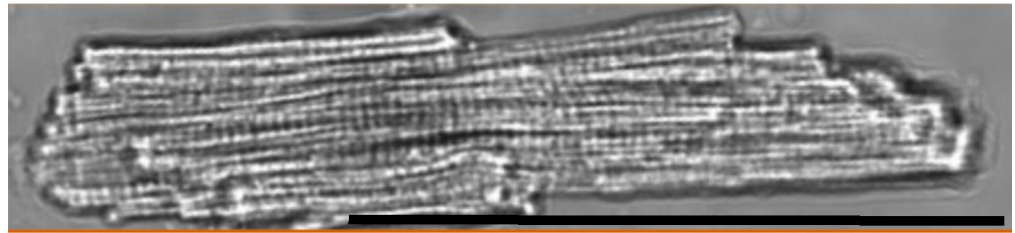


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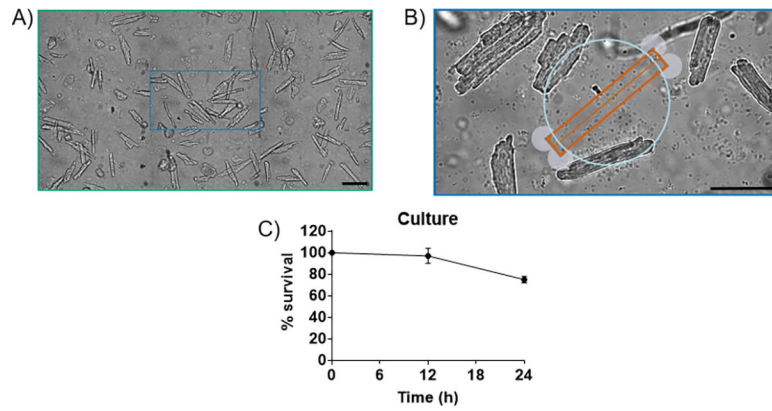
A)



B)

**Figure 1.**

A) 4-month-old C57Bl/6 mouse cardiomyocytes imaged using Ionoptix Multicell bright field. Yield ~80%, 20X magnification. B) Ionoptix Multicell bright field image of a 4-month-old C57Bl/6 mouse cardiomyocyte. Cardiomyocyte are quiescent, exhibiting rod-shape with no membrane blebs. Scale bar is 100  $\mu\text{m}$ .



**Figure 2.**

A) 4-month-old C57Bl/6 mouse cardiomyocytes cultured for 24 hrs in M199 media. ~80% viability, 20X magnification. B) Myocytes cultured for 24h. At 24h, cardiomyocytes exhibit rod-shape with no membrane blebs. C) % survival curve for cardiomyocytes cultured for 24h using the described method. Scale bar is 100  $\mu$ m.

**Table 1.**

A table of noted differences between our method, Langendorff methods, and previously published Langendorff-free methods of isolating mouse cardiomyocytes.

	<b>Our adaptations</b>	<b>Langendorff technique</b>	<b>Langendorff-free method</b>
Prevention of blood clots	3 mL ice-cold clearing solution (contains EDTA)	Heparin	17 mL of EDTA solution
Non-perfusion time	Shortest	Longest	Short, but does not use ice-cold solution
Technical skill/training	Little	High	Little
Supporting platform to stabilize needle	Yes		No
Temperature control of buffers (37°C)	Yes	Yes	No
Enzyme	Liberase	Lab-dependent	Collagenase
Post-digestion	Yes	Lab-dependent	No

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## table-materials

Name of Material/Equipment	Company	Catalog Number	Comments/ Description
10 cc Bd Luer-Lok Syringe	Fisher Sci	14-827-52	
10 mL Pyrex Low-Form Beaker	Cole-Palmer	UX-34502-01	
100 mL polypropylene cap glass media storage bottle	DWK Life Sciences	UX-34523-00	
14 mL Round-Bottom Polypropylene Test Tubes With Cap	Fisher Sci	14-959-11B	
2,3-Butanedione Monoxime	Sigma	B0753	>98%
3 cc BD Luer-Lok Syringe	Fisher Sci	14-823-435	
35 mm glass bottom dishes	MatTek Corporation	P35G-1.0-20-C	
50 mL BD Syringe without Needle	Fisher Sci	13-689-8	
50 mL Conical Centrifuge Tubes	Cole-Palmer	EW-22999-84	
95% O2 5% CO2			
AIMS Space Gel Heating Pad	Fisher Sci	14-370-223	
BD PRECISIONGLIDE™ 27 G X 1/2" HYPODERMIC NEEDLES	Becton Dickinson	305109	
Bovine Serum Albumin	Sigma	A3803	Heat shock fraction, lyophilized powder, essentially fatty acid free, >98%
Calcium Chloride dihydrate	Sigma	C7902	>99%
D-(+)-Glucose	Sigma	G7021	Suitable for cell culture, >99.5%
DMEM	Fisher Sci	11965092	
EDTA	Fisher Sci	AAA1071336	
Falcon® 100 mm TC-treated Cell Culture Dish	Corning	353003	
FBS	R&D Systems (Bio-technie)	S11195	
Fisherbrand Isotemp Heated Immersion Circulators	Fisher Sci	13-874-432	
Hartman Mosquito Hemostatic Forceps	World Precision Instruments	15921	
Hausser Scientific™ Hy-Lite™ Counting Chamber Set	Fisher Sci	02-671-11	
HEPES	Sigma	H4034	>99.5%
Labeling Tape	Fisher Sci	15-901-10R	
Legato 100 Syringe Pump	kdScientific	788100	
L-glutathione	Fisher Sci	ICN19467980	
Liberase TH Research Grade	Sigma	5401135001	High thermolysin concentration
M199	Fisher Sci	MT10060CV	
Magnesium Chloride	Invitrogen	AM9530G	
Mouse Laminin	Corning	354232	
Pen/Strep	Fisher Sci		
Potassium Chloride	Sigma	P5405	>99%
Precision Digital Reciprocating Water Bath	ThermoFisher Scientific	TSCIR19	
Sodium Bicarbonate	Sigma	S5761	Suitable for cell culture
Sodium Chloride	Sigma	S5886	>99%
Sodium phosphate monobasic	Sigma	S5011	>99%

<b>Name of Material/Equipment</b>	<b>Company</b>	<b>Catalog Number</b>	<b>Comments/ Description</b>
Sterile Cell Strainer 70 um	Fisher Sci	22-363-548	
Student Fine Scissors	Fine Science Tools	91460-11	
VWR Absorbent Underpads	Fisher Sci	NC9481815	

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 Perfusion Pump Settings

Volume	49.99 mL
Rate	120.6 mL/h
Diameter	26.60 mm

## Clearing Solution

Compound	MW (g/mol)	Concentration (mM)	g/L
NaCl	58.44	130	7.5972
KCl	74.55	5	0.37275
NaH <sub>2</sub> PO <sub>4</sub>	119.98	0.5	0.05999
HEPES	238.3	10	2.383
Glucose	180.16	10	1.8016
BDM	101.1	10	0.0101
EDTA	292.24	5	1.4612

Make in 1 L ultrapure 18.2 MΩ\*cm H<sub>2</sub>O. Sterile Filter, pH 7.4

## Perfusion Buffer

Compound	MW (g/mol)	Concentration (mM)	g/L
NaCl	58.44	130	7.5972
KCl	74.55	5	0.37275
NaH <sub>2</sub> PO <sub>4</sub>	119.98	0.5	0.05999
HEPES	238.3	10	2.383
Glucose	180.16	10	1.8016
BDM	101.1	10	0.0101
MgCl <sub>2</sub>	95.2	1	0.095

Make in 1 L ultrapure 18.2 MΩ\*cm H<sub>2</sub>O. Sterile Filter, pH 7.4

## Digestion Buffer

Compound	MW (g/mol)	Concentration (mM)	g/L
NaCl	58.44	130	7.5972
KCl	74.55	5	0.37275
NaH <sub>2</sub> PO <sub>4</sub>	119.98	0.5	0.05999
HEPES	238.3	10	2.383
Glucose	180.16	10	1.8016
BDM	101.1	10	0.0101
MgCl <sub>2</sub>	95.2	1	0.095
CaCl <sub>2</sub>	110.98	0.025	2.7745

Liberase TH 770 ul

Make in 1 L ultrapure 18.2 MΩ\*cm H<sub>2</sub>O. Sterile Filter, pH 7.4

## Stop Buffer

Stop Buffer = Perfusion Buffer + 2% BSA

Compound	MW (g/mol)	Concentration (mM)	g/L
NaCl	58.44	130	7.5972
KCl	74.55	5	0.37275
NaH <sub>2</sub> PO <sub>4</sub>	119.98	0.5	0.05999



HEPES	238.3	10	2.383
Glucose	180.16	10	1.8016
BDM	101.1	10	0.0101
MgCl <sub>2</sub>	95.2	1	0.095

Bovine Serum Albumin 66463

Make in 1 L ultrapure 18.2 MΩ\*cm H<sub>2</sub>O. Sterile Filter, pH 7.4

M199 Media

Compound	MW (g/mol)	Concentration (mM)	g/L
NaHCO <sub>3</sub>	84.007		2.2
HEPES	238.3		2.6
L-glutathione	307.3235		3.073
BDM	101.1		
BSA	66463		0.2

M199 Media

Pen/Strep 10 ml

Make in 1 L ultrapure 18.2 MΩ\*cm H<sub>2</sub>O. Sterile Filter, pH 7.4

DMEM Media

Compound	Volume (mL)
DMEM	45.5
FBS	2.5
Pen/Strep	0.5
BDM	1

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