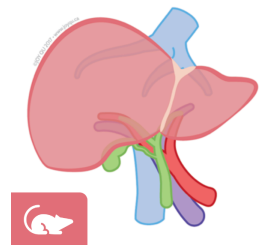


Nov 24, 2020

Mouse 2- Step Collagenase Liver Perfusion protocol

DOI

dx.doi.org/10.17504/protocols.io.53qg8mw



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Manuscript citation:



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Protocol status: Working

We use this protocol and it's working

Created: August 02, 2019

Last Modified: November 24, 2020


Protocol Integer ID: 26448

Keywords: step collagenase perfusion protocol, optimal cell recovery from the tissue, mouse liver, difficulty in cell culture, cell culture, optimal cell recovery, primary cell culture, cell analysis, fragile cell population, single cell, enzymatic technique, cell

Abstract

Analyzing the mouse liver requires optimal cell recovery from the tissue in terms of quality (viability) and quantity. Harsh dissociation methods can damage fragile cell populations, resulting in low viability and difficulty in cell culture. We have developed a gentle surgical and enzymatic technique based on the two-step collagenase perfusion protocol for single-cell analysis and primary cell culture.

Protocol materials

 Dulbecco's Modified Eagle's Medium - high glucose **Merck MilliporeSigma (Sigma-Aldrich) Catalog #D5796**

 Heparin LEO® **LEO Pharma Catalog #006174-09**

 Collagenase from Clostridium histolyticum **Merck MilliporeSigma (Sigma-Aldrich) Catalog #C5138**

Troubleshooting



Preparation

- 1 **Anesthetize** mouse with inhalational anesthesia (4-5% isoflurane) in the induction chamber
- 2 **Heparinize** the animal intraperitoneally by injecting [M] 5 units/g (body weight) of heparin:

Note

Preventing blood clotting improves cell recovery.

 Heparin LEO® LEO Pharma Catalog #006174-09

- 3 **Once the animal is anesthetized, transfer** it onto a homeothermic warming pad. The animal will receive 1-3% isoflurane via face-mask inhalation for the remaining steps.
- 4 **Prepare the abdomen for surgery:** shaved with a trimmer, prepared with iodine-based solution and alcohol-based solution, and draped in a sterile fashion.

Laparotomy

- 5 **Midline incision:** cut open the abdomen from the pubic symphysis to the xiphoid process. Flip over the intestines to locate the liver and its portal vein.

Please refer to Figure 14.4.1 of the following publication:

Citation

Froh M, Konno A, Thurman RG (2003). Isolation of liver Kupffer cells..
Current protocols in toxicology.

<https://doi.org/10.1002/0471140856.tx1404s14>

LINK

Cannulation

- 6 **Cannulate** the portal vein with the 22-gauge catheter. Secure it cannulation by suture or a micro serrefine.

Equipment	
Insyte™ Autoguard™ BC	NAME
Intravenous Catheter	TYPE
BD	BRAND
382523	SKU
https://www.bd.com/en-ca/offerings/capabilities/infusion-therapy/iv-catheters/bd-insyte-autoguard-bc-shielded-iv-catheter-with-blood-control-technology	LINK
Non-winged, blue, 22 gauge, 1 inch, 0.9 × 25 mm	SPECIFICATIONS

Equipment

Schwartz Micro Serrefine

NAME

Micro Serrefine

TYPE

Fine Science Tools

BRAND

18052-03

SKU

<https://www.finescience.com/en-US/Products/Vascular-Instruments/Clamps-Occluders/Schwartz-Micro-Serrefines/18052-03>

LINK

Sharp Bend

SPECIFICATIONS





7 **Collect blood:** collect blood from the inferior vena cava (IVC) with a 20-gauge needle.



8 **Cut the IVC and proceed to the next step immediately.** Cutting the IVC allows blood and perfusate to drain.

Perfusion

9 **Perfuse liver with calcium-free buffer without collagenase:**  10 mL of Hanks' Balanced Salt Solution (HBSS) **without** calcium or magnesium +  0.5 millimolar (mM) EGTA at a flow rate of **2 mL/min** until fluid drained from the IVC is no longer red (containing blood). This is done with a pump with no recirculation.

5m



Note

This flow rate (**2 mL/min**) is slower than other liver perfusion protocols. We found that a slower perfusion rate results in hepatocytes with higher quality and quantity and therefore suitable for analyses such as single-cell RNA sequencing.



Equipment

Model EP-1 Econo Pump

NAME

Peristaltic Pump

TYPE

Bio-Rad

BRAND

7318140



SKU

<http://www.bio-rad.com/en-ca/product/model-ep-1-econo-pump?ID=6ee41e9b-5ac5-449c-ae7d-578dca8ed6cf>

LINK



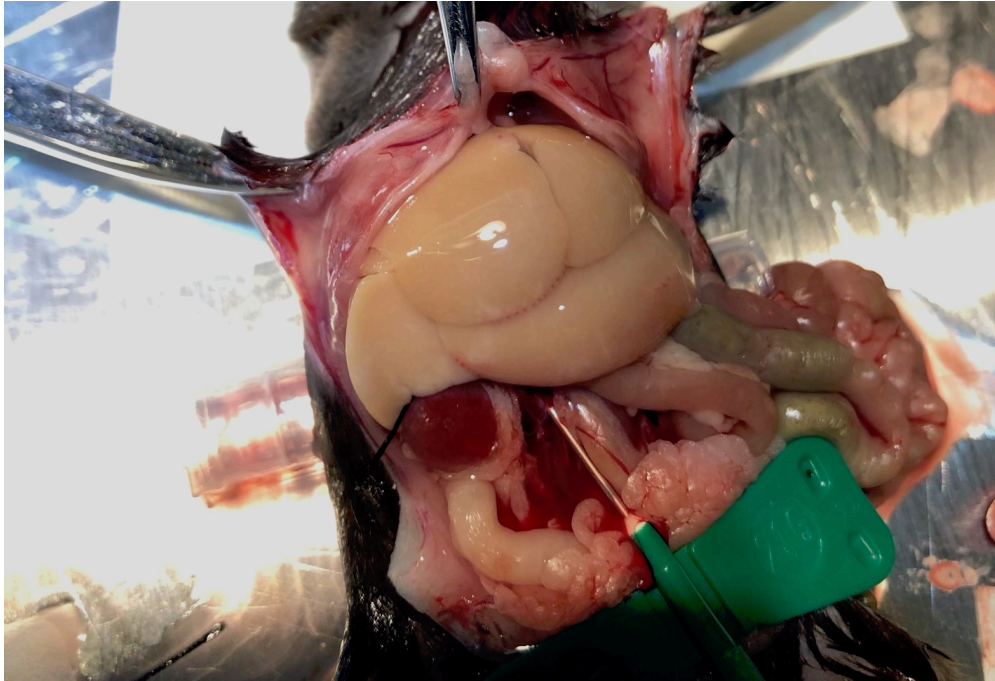
10

Perfuse liver with calcium-containing buffer with collagenase:  10 mL of Hanks' Balanced Salt Solution (HBSS) **with** calcium and magnesium +  0.1 mg/mL type IV collagenase at the same flow rate (**2 mL/min**). This is done with the same pump with no recirculation.

5m



Collagenase from Clostridium histolyticum **Merck MilliporeSigma (Sigma-Aldrich) Catalog #C5138**



A well-perfused liver shows drastic discoloration due to the lack of blood. A well-dissociated liver shows white patches.

Harvest

- 11 **Excise** the dissociated liver out of the animal and place it in DMEM containing 10% FBS, which inactivates the collagenase enzyme.



Dulbecco's Modified Eagle's Medium - high glucose **Merck MilliporeSigma (Sigma-Aldrich)** Catalog #D5796

Cell Isolation

- 12 **Gently cut** the excised liver with a scalpel to release the dissociated cells.

Note

Good dissociation can be visualized by abundance of cells released (in pink) with little cutting. Shake the tissue gently to release cells. Avoid tearing the tissue with the scalpel (horizontal movement); only cut the tissue vertically.



13 **Filter** dissociated cells with a 70 µm filter:

Equipment

70 µm Cell Strainer

NAME

Cell Strainer

TYPE

Falcon

BRAND

352350

SKU

<https://ecatalog.corning.com/life-sciences/b2c/US/en/Cell-Culture/Cell-Culture-Accessories/Cell-Strainers/Falcon®-Cell-Strainers/p/352350?clear=true>

LINK

White, Sterile, Individually Packaged


SPECIFICATIONS



Cell Enrichment

14 **Enrich dissociated cells for hepatocytes** by centrifugation 50 x g


5m

 Room temperature for 5 minutes. The supernatant can be further purified in the next step. Resuspend the pellet (hepatocyte-rich) with DMEM.



15 **Enrich supernatant for non-parenchymal cells (NPC)** by centrifugation 400 x g

5m

 Room temperature for 5 minutes. Discard the supernatant. Resuspend the pellet (NPC-rich) with DMEM.





Citations

Step 5

Froh M, Konno A, Thurman RG. Isolation of liver Kupffer cells.

<https://doi.org/10.1002/0471140856.tx1404s14>