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## Mouse 2- Step Collagenase Liver Perfusion protocol

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1

Works for me

[dx.doi.org/10.17504/protocols.io.53qg8mw](https://dx.doi.org/10.17504/protocols.io.53qg8mw)



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### 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.

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### 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.

### Preparation

1 **Anesthetize** mouse with inhalational anesthesia (4-5% isoflurane) in the induction chamber

2

**Heparinize** the animal intraperitoneally by injecting **100** **5 units/g (body weight)** of heparin:

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% isofluorane 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:*

Froh M, Konno A, Thurman RG (2003). Isolation of liver Kupffer cells.. Current protocols in toxicology.  
<https://doi.org/10.1002/0471140856.tx1404s14>

#### Cannulation

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

Insyte™ Autoguard™ BC  
Intravenous Catheter

BD 382523 

Non-winged, blue, 22 gauge, 1 inch, 0.9 × 25 mm



Schwartz Micro Serrefine  
Micro Serrefine

Fine Science Tools 18052-03 [↗](#)  
Sharp Bend



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

5m

9

**Perfuse liver with calcium-free buffer without collagenase:** **10 mL** of Hanks' Balanced Salt Solution (HBSS) **without** calcium or magnesium + **0.5 Milimolar (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.

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.

Model EP-1 Econo Pump  
Peristaltic Pump

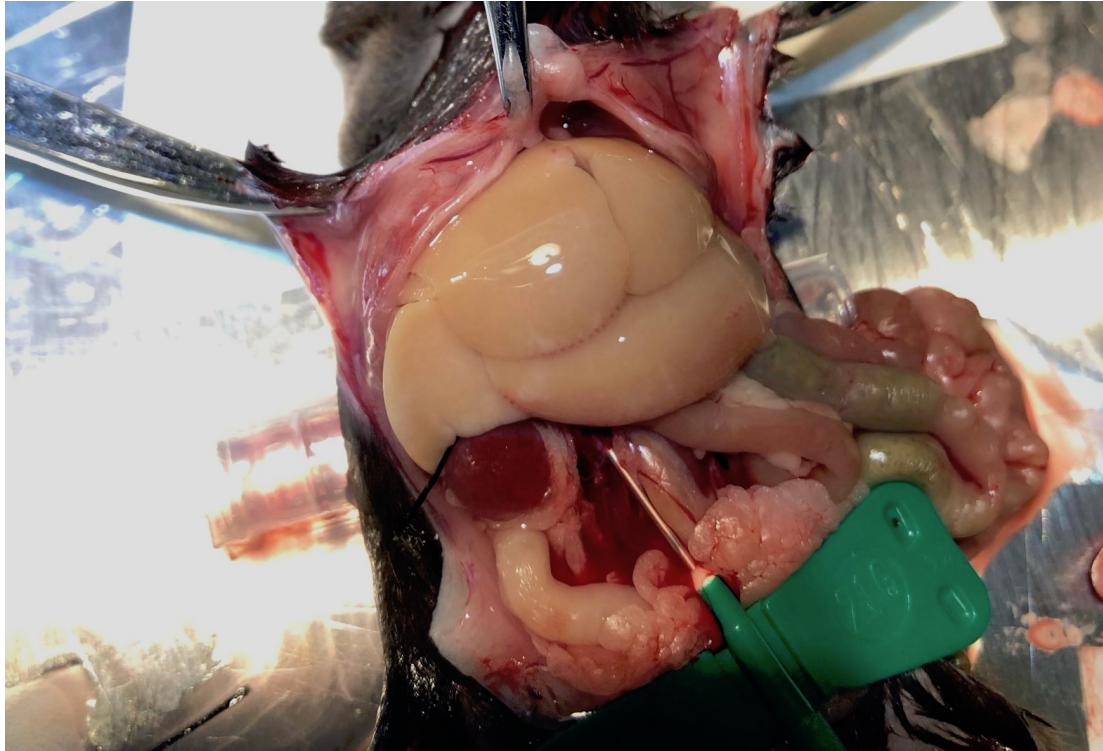
Bio-Rad 7318140 [↗](#)



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.

[Collagenase from Clostridium histolyticum](#) **Millipore**

**Sigma 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](#) **Millipore**

**Sigma Catalog #D5796**

## Cell Isolation

- 12

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

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  $\mu$ m filter:

70 µm Cell Strainer

Cell Strainer

Falcon

352350



White, Sterile, Individually Packaged



#### Cell Enrichment

14



5m

**Enrich dissociated cells for hepatocytes** by centrifugation **50 x g** **Room temperature** for 5 minutes. The supernatant can be further purified in the next step. Resuspend the pellet (hepatocyte-rich) with DMEM.

15



5m

**Enrich supernatant for non-parenchymal cells (NPC)** by centrifugation **400 x g** **Room temperature** for 5 minutes. Discard the supernatant. Resuspend the pellet (NPC-rich) with DMEM.