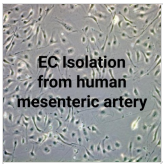

Sep 26, 2020

EC isolation from human mesenteric artery for scRNA-seq

Xiaofang Tang[1], Kiran KS Sriram[1], Yingjun Luo[1], Zhen Chen[1]

[1]City of Hope

1 *Works for me* This protocol is published without a DOI.

Xiaofang Tang

ABSTRACT

This protocol outlines a method to efficiently isolate the human mesenteric arterial endothelial cells. Following the physical scraping, endothelial cells from the inner surface of the vessels are separately liberated using a digestion buffer solution. The procedure takes approximately one hour and has been validated for down-stream applications such as FACS, VE-cad immunostaining and single cell RNA profiling.

PROTOCOL CITATION

Xiaofang Tang, Kiran KS Sriram, Yingjun Luo, Zhen Chen 2020. EC isolation from human mesenteric artery for scRNA-seq. **protocols.io** https://protocols.io/view/ec-isolation-from-human-mesenteric-artery-for-scrn-bf37jqrn

KEYWORDS

endothelial cells, isolation, EC isolation, human, mesenteric artery, human mesenteric artery, scRNA-seq

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GUIDELINES

Cells should be washed and resuspended in LoBind 2.0 mL eppendorf tubes and wide-bore pipette tips for better cell viability.
Pipette the cell suspension very gently and slowly for all steps involved.
Each 4-5 cm vessel, use **1 mL TrypLE** .
Keep the tissues/cells **On ice** .

MATERIALS

NAME	CATALOG #	VENDOR
DPBS (10X), calcium, magnesium	14080055	Thermo Fisher
Medium 199	M2520-10X	Sigma Aldrich
40 µm cell strainer	14100150	Fisher Scientific
Trypan Blue Solution	MT25900CI	Corning
Disposable Safety Scalpels	6008TR-10	Myco Instrumentation
Fisher BioReagents™ Bovine Serum Albumin Heat Shock Treated	BP1600-100	Fisher Scientific

NAME	CATALOG #	VENDOR
Pipette Tips RT LTS 1000µL LW 768A/8	30389219	Rainin
DNA LoBind Tube 2.0 mL	022431048	Eppendorf
Pipet-Lite Pipette Unv. SL-1000XLS	17014407	Rainin
30 G X 1 in Needle	305128	BD Biosciences
Pipette Tips RT LTS 1000µL FLW 768A/8	30389218	Mettler Toledo
TrypLE™ Express Enzyme (1X) phenol red	12605010	Thermo Fisher Scientific

STEPS MATERIALS

NAME	CATALOG #	VENDOR
Pipette Tips RT LTS 1000µL FLW 768A/8	30389218	Rainin

MATERIALS TEXT

Additional materials required:

- 10-cm petri-dish
- 5 mL tubes
- Sterile surgical tools
- Candle platform

- 0,04% BSA-DPBS

SAFETY WARNINGS

For hazard information and safety warnings, please refer to the SDS (Safety Data Sheet).

BEFORE STARTING

Warm up **1 mL TrypLE** at **37 °C** for **00:05:00** before use.

Prepare at least **6 mL 0.04% BSA-DPBS** .

EC isolation from human mesenteric artery for scRNA-seq

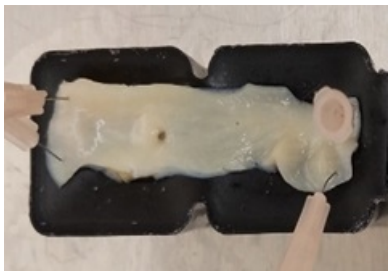
1

Wash freshly isolated human mesenteric artery (~5 cm in length) with DPBS in a 10-cm petri-dish.
Use **sterile surgical tools** to clean the vessel (Removing the fat and outer connective tissues).

Freshly isolated human mesenteric artery.

2 Cut open the vessel and expose the inner lumen. Anchor the four corners of the vessel using 23G needles on a black

wax.



Anchored vessel.

3

Add **1 mL TrypLE** to the lumen side of the artery.

4 Use a scalpel to scrape the lumen 10-15 times (the purpose is to dissociate the endothelium of the intima).

5

Keep the remaining vessel in DPBS **On ice** . The rest of the vessel can be scraped again to collect the vascular smooth muscle cells in the intima layer.

6

Transfer **1 mL cell suspension** to a 5 mL tube.

7

Incubate with shaking at ~ **150 rpm, 37°C** for **00:05:00** .

8

Add equal volume (**1 mL**) of M199 medium (or any EC growth media) to stop enzyme digestion.

9

Centrifuge at **600 x g, 4°C** for **00:05:00** .

10

Remove the supernatant and re-suspend the pellet gently and thoroughly with **1 mL 0.04% BSA-DPBS** using wide-orifice LiteTouch pipette tips.

Pipette Tips RT LTS 1000µL FLW 768A/8
by Rainin
Catalog #: 30389218

11

Pass the cell suspension through a 40 µm strainer to remove any cell debris. Wash the strainer by adding another **4 mL 0.04% BSA-DPBS** .

12

Centrifuge at **600 x g, 4°C** for **00:05:00** .

13

Remove the supernatant and re-suspend the pellet with **0.5 mL 0.04% BSA-DPBS** by using wide-orifice LiteTouch pipette tips and gently pipetting up and down along the inner wall of the tubes.

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14

Count the cell viability:
Mix **10 µl trypan blue** + **10 µl cell suspension** . Take **10 µl mixture** to count cell number.

Ensure there's no cell debris, significant number of doublets or cell clumps in the suspension.

Always keep the cells **On ice** .

Usually we could get at ~ **10^5 cells in total**.

15

The EC isolation results.

2.png

Morphology of isolated mesenteric arterial endothelial cells after two passages (Passages 2 and 3).

flow-1.png

CD31 flow cytometry of isolated mesenteric arterial ECs, HUVEC was used as a positive control

16 Use appropriate number of cells for scRNA library preparation following the *10XGenomics 3' Expression protocol*. The rest of the cells can be cultured or used for other downstream assays.