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Adult mouse liver dissociation (on ice) V.2

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Human Cell Atlas Metho...

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Protocol status: Working
We use this protocol and it's working

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Abstract

This protocol is used to dissociate adult (10 week) CD-1 mouse liver "on ice", preserving authentic gene expression profiles. The first layer consists of 3.5 hours rotation at 4 °C in 0.25% trypsin, and the second layer consists of 15 min. in *bacillus licheniformis*, with trituration on ice. The yield is 5000 cells/mg with 97% viability (as measured by trypan blue).

Attachments

DOCX

Liver cell dissociat...

17KB

Guidelines

Trypsin Enzyme Mix
500 µL DPBS (ThermoFisher cat. # 14190) / BSA 0.1% (0.05% BSA/DPBS final conc.)
500 µL Trypsin 0.5%/EDTA – 0.25% final conc. (ThermoFisher, cat. # 15400054)

***Bacillus Licheniformis* Enzyme Mix**
899 µL DPBS (ThermoFisher, cat. # 14190)
1 µL 0.5 M EDTA (0.5 mM final) (Sigma, A8806)
100 µL *Bacillus Licheniformis* 100 mg/mL - 10 mg/mL final conc. (Sigma, P5380)

Preparing enzymes:
The *bacillus licheniformis* is made up in DPBS (#14190). It is aliquoted and stored at -80 ºC at 100 mg/mL in 100 µL aliquots.
The trypsin is aliquoted and stored at -20 ºC.

Required Equipment & Consumables:
Refrigerated centrifuge
Fisher tube rotator, cat. # 88-861-051 (or similar rotation device)
Pipettes and pipet tips (MLS)
15, 50 ml Conicals (MLS)
1.5 mL tubes (MLS)
30 µM filters (MACS SmartStrainers, 130-098-458)
Petri dishes (MLS)
Razor blades (MLS)
Ice bucket w/ice (MLS)
Hemocytometers - InCyto Neubauer Improved (DHC-NO1-5)

Required reagents:
Red Blood Cell Lysis Buffer - Sigma (R7757)

The protocol workflow is as follows:
1. Isolate liver
2. First layer
3. Second layer
4. Preparing cells for Chromium/DropSeq

Materials

MATERIALS

RBC Lysis Buffer **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**R7757

Troubleshooting

Before start

-Prepare enzyme mixes and leave on ice.
-Cool centrifuges to 4 °C.
-Isolate and transport tissue in ice-cold DPBS.

Isolate tissue

1 Dissect out liver tissue and immerse/transport in ice-cold PBS.

2 Using sterile forceps, place tissue on petri dish on ice. Remove excess DPBS using pipet. Mince tissue thoroughly on petri dish on ice (~2 min) until fine paste. Manipulate tissue with forceps while mincing with razor blade.
00:02:00 mince on ice

First layer of dissociation

3 Add 18 mg tissue to 1 mL trypsin enzyme mix. Shake tube vigorously to re-suspend tissue.
18 mg minced tissue

4 Leave digest mix rotating at 4 °C for 3.5 hrs on Fisher tube rotator. Every 45 min (4X total), stop rotation briefly and shake tube vigorously to re-suspend tissue.
03:30:00 rotate at 4 °C 00:45:00 shake vigorously

5 After 3.5 hours, transfer digest mix to 15 mL conical & add 10 mL ice-cold DPBS to dilute trypsin mix. Re-suspend digest mix.
10 mL ice-cold DPBS

6 Spin 300 g for 5 min at 4 °C to pellet cells and tissue chunks. Remove supernatant.
00:05:00 spin 300 g 4 °C

7 Re-suspend cells and tissue in 1 mL ice-cold DPBS and transfer to 1.5 mL tube. Let chunks settle one minute on ice (released cells should remain in supernatant).
00:01:00 let chunks settle on ice

8 After tissue chunks have settled to bottom of tube, transfer 75% of supernatant, containing released cells onto 30 µM filter on 50 mL conical. Rinse filter with 6 mL ice-cold PBS/BSA 0.04%. Save filter and flow-through for next steps.
750 µL pipet off supernatant 6 mL ice-cold PBS/BSA 0.04%

Second layer of dissociation

9 To tissue clumps at bottom of tube, add 1 mL bacillus licheniformis enzyme mix. Triturate 10X.

1 mL bacillus licheniformis enzyme mix

10 Continue incubating on ice in bacillus licheniformis enzyme mix. Shake every minute and triturate 10X every 2 min for 15 additional minutes (3 hr. 45 min total time) until the majority of clumps are broken up.

00:15:00 incubate on ice | 00:02:00 triturate 10x every 2 min

11 Transfer entire volume of digest mix to the same 30 µM filter. Rinse filter with 5 mL ice-cold PBS/BSA 0.04%.

5 mL ice-cold PBS/BSA 0.04%

12 Re-suspend and transfer flow-through containing cells to 15 mL conical.

13 Spin 300 g for 5 min at 4 °C. Remove supernatant (down to ~100 µL) and leave in 15 mL conical.

00:05:00 300 g for 5 min | 4 °C

RBC Lysis

14 Add 1 mL RBC lysis buffer; triturate 20X; let sit 2 min. on ice.

1 mL RBC lysis buffer

00:02:00 incubate on ice

15 After 2 min., add 5 mL ice-cold PBS/BSA 0.04% and re-suspend cells.

5 mL ice-cold PBS/BSA 0.04%

16 Add re-suspended cells to new 30 µM filter on 50 mL conical; rinse filter with 7 mL ice-cold PBS/BSA 0.04%. Transfer flow-through to 15 mL conical.

7 mL ice-cold PBS/BSA 0.04%

Preparing for single cell analysis

17 Spin 1800 RPM (low-speed spin) for 3 minutes at 4 °C to pellet cells and leave the majority of debris in supernatant.

00:03:00 spin 1800 RPM | 4 °C

18 Remove supernatant; re-suspend cells in ~100 µL ice-cold PBS/BSA 0.04% and analyze using hemocytometer with trypan blue. Adjust cell concentration to 1,000 cells/µL for Chromium or 100 cells/µL for DropSeq.

100 µL ice-cold PBS/BSA 0.04%