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Adult mouse skin dissociation protocol (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 was developed to dissociate adult (8-10 wk) mouse skin "on ice". It utilizes two layers of digestion with a *Bacillus licheniformis* protease cocktail, combined with mechanical disruption from a dounce homogenizer. The cell yield is 3000 cells/mg.

Attachments

PDF

Skin adult mouse dis...

99KB

Guidelines

***Bacillus Licheniformis* Enzyme Mix (1 mL per 23 mg tissue):**
100 µL b. lich 100 mg/mL (10 mg/mL final) (Sigma, P5380)
1 µL 0.5 M EDTA (Sigma, A8806)
899 µL DPBS (no Ca, Mg) ThermoFisher (cat. #14190)

Preparing enzymes:
The enzyme is made up in DPBS (#14190). It is aliquoted and stored at -80 °C at 100 mg/mL in 100 µL aliquots..

Reagents
Enzymes, trypsin inhibitor, BSA and DNAse are made up in DPBS (no Ca, no Mg) from Thermo Fisher (14190).
Bovine Serum Albumin - Sigma (A8806).
Hypothermosol FRS

Required supplies:
2 mL dounce homogenizer – Bellco (1984-10002)
Centrifuge for 1.5 mL, 15 mL conicals
Pipettes and pipet tips
15 ml Conicals (MLS)
1.5 mL tubes (MLS)
30 µM filters - Miltenyi (130-098-458)
Petri dishes (MLS)
Razor blades (MLS)
Ice bucket w/ice
Hemocytometers - InCyto Neubauer Improved (DHC-NO1-5)

Isolating tissue

1 After euthanizing mouse, remove hair using Nair: dab with Nair, wait 30 secs, wipe with wet paper towel.

2 Isolate tissue and place in ice-cold hypothermosol.

3 Scrape off underlying layer of fatty / connective tissue using scalpel before proceeding.

4 Mince skin tissue thoroughly on petri dish on ice for 3-4 min on ice into 1-mm3 pieces using razor blade while manipulating tissue with forceps – you will need to use grinding motion and vigorously break up tissue.
00:04:00 mincing

1st digest layer

5 Place 23 mg minced tissue into 1 mL B. Lich enzyme cocktail. Incubate on ice.
23 mg minced tissue

6 Shake every min; triturate 10x every 2 min with p1000 w/tip cut (start triturating at 2 min) for 20 min.
00:20:00 digest
00:01:00 shake 00:02:00 triturate 10X

7 After 20 mins of triturating on ice, use pipet to transfer digest mix to 2 mL dounce homogenizer. Use 10 strokes of Pestle A every 2 min (4 series total, 8 min). Digest mix should become turbid.
00:02:00 dounce homogenize 00:08:00 digest using dounce

8 Transfer back to 1.5 mL tube using 1 mL serological pipet. Mix thoroughly and allow to settle on ice 2 min.

00:02:00 settle on ice

9 Save 70% (700 µL) of supernatant, leaving chunks at the bottom of the tube; apply to 30 µM filter on 15 mL conical. Rinse filter w/5 mL ice-cold PBS/BSA 0.04%. Save flow through on ice and keep filter on tube for 2nd layer.

700 µL save supernatant | 5 mL rinse filter w/ice-cold PBS/BSA 0.04%

2nd digest layer

10 Add additional 1 mL b. Lich enzyme mix to residual tissue chunks.

1 mL b. lich enzyme mix

11 Triturate 10x every 2 min, shake every min while incubating on ice for 20 additional mins. (50 min. total digest time).

00:20:00 additional digest time

00:02:00 triturate 10x | 00:01:00 shake every min.

12 Transfer entire volume to same 30 µM filter on 15 mL conical. Rinse with additional 5 mL ice-cold PBS/BA 0.04%.

5 mL ice-cold PBS/BSA 0.04%

Preparing cells for single cell analysis

13 Centrifuge at 300 g for 5 min at 4 °C. Remove supernatant & re-suspend in 100 µL PBS/BSA 0.04%. Examine using hemocytometer with trypan blue.

00:05:00 centrifuge at 300 g | 100 µL re-suspend in ice-cold PBS/BSA 0.04%

14 Adjust concentration to 1,000 cells/µL for Chromium or 100 cells/µL for DropSeq.