Procedure

Semi-automated enzymatic digestion using the dissociation enzyme mix D (kidney)

Introduction

These guidelines provide recommendations for performing low-temperature enzymatic dissociation for single-cell workflows and cell counting operations using the Omics Bundle. They include instructions for using VIA Extractor™ tissue disaggregator, VIA Freeze™ Uno controlled-rate freezer, Omics Pouch, and Omics Clamp.

Required materials

The following materials are typically required in combination with the dissociation enzyme mix D for kidney tissue dissociation.

Source	Material
Provided in dissociation enzyme mix D (kidney)	• Enzyme 1 • Enzyme 2 • Buffer A
Equipment provided by the user	• VIA Extractor tissue disaggregator • VIA Freeze Uno controlled-rate freezer • Omics Clamp • Centrifuge with refrigeration • Heat sealer • Micro pipettes • Pipette controller • Syringe
Consumables provided by the user	• Mouse kidney tissue • Omics Pouch • 50 mL sterile conical centrifuge tubes • 5 mL Luer-Lok Syringe • 100 µm, 70 µm, and 40 µm cell strainers for 50 mL centrifuge tubes • 1000 µL wide-bore pipette tips • 25 mL, 10 mL, and 5 mL serological pipettes • Red blood cell lysis buffer • Water for cell culture • Refrigerated Dulbecco's phosphate-buffered saline (DPBS) • 0.5 M EDTA solution • Micropipette tips • Bovine serum albumin (BSA) • Refrigerated fetal bovine serum (FBS) • Sterile 25 mL reservoir

Preparation

Prechill the centrifuge to 4°C, and prepare the following reagents:

- DPBS with 10% (v/v) FBS. Put on ice.
- DPBS/BSA/EDTA solution: 50 mL DPBS with 0.4% (w/v) BSA (200 mg) and 10 µL 0.5 M EDTA prepared newly for each procedure. Put on ice.
- 15 mL 1× red blood cell lysis buffer per sample, prepared from 10× red blood cell lysis buffer and water for cell culture. Keep at room temperature.
- Dissociation enzyme mix D (kidney) in a sterile reservoir in an appropriate volume for the sample size according to the table below:

Note: One sample is a single mouse kidney weighing up to 300 mg.

Reagent	Volume for 1 sample	Volume for 2 samples	Volume for 3 samples
Enzyme 1	1 µL	2 µL	3 µL
Enzyme 2	275 µL	550 µL	825 µL
Buffer A	5.224 mL	10.448 mL	15.672 mL
Total volume	5.5 mL	11.0 mL	16.5 mL

Dissociation protocol

Follow the steps below to efficiently dissociate the tissue sample using the VIA Extractor tissue disaggregator. Keep your samples on ice and your reagents cold during the protocol to reduce cellular aggregation.

Note: After 15 minutes of dissociation, most of the tissue will be dissociated. Extending the dissociation time will dissociate tissue further but will increase cellular aggregation.

1. Enter the following settings for the dissociation protocol. Refer to the VIA Extractor tissue disaggregator Operating Instructions (29427281) and the Omics Bundle Quick Start Guide (29657648) for details on setting a protocol.
   - Speed: 200 rpm
   - Temperature: 2°C
   - Time: 15 minutes

   Note: The VIA Freeze Uno controlled-rate freezer temperature must be set to 2°C to allow the VIA Extractor tissue disaggregator to cool to 4°C.
2. Wait 30 minutes for the VIA Extractor tissue disaggregator to cool to 4°C.
   Note: The VIA Freeze Uno controlled-rate freezer temperature must be set to 2°C to allow the VIA Extractor tissue disaggregator to cool to 4°C.
3. Add one tissue sample weighing up to 300 mg using the syringe and 5 mL of enzyme mixture as the digestive solution with a Luer-Lok syringe into each compartment of the Omics Pouch. Refer to the Omics Pouch Instructions for Use (29492826) and the heat seal process for the Omics Pouch for details on how to load the tissue sample and enzyme mixture into the Omics Pouch.
4. Run the protocol on the VIA Extractor tissue disaggregator.

Prepare for post-dissociation processing

While the VIA Extractor tissue disaggregator protocol is running, perform the following preparation steps:

1. Place one 50 mL conical centrifuge tube per sample on ice, with a 100 µm cell strainer attached on top.
2. Wet each cell strainer with 1 mL of 10% FBS in DPBS.

Red blood cell lysis and sample straining

Once the dissociation run has finished on the VIA Extractor tissue disaggregator, remove the Omics Pouch from the Omics Clamp and proceed to red blood cell lysis and sample straining.

Note: To reduce cellular aggregation, perform the post-dissociation steps swiftly. Keep all reagents and samples on ice, except for the red blood cell (RBC) lysis solution.

1. Extract the cell suspension from the Omics Pouch. Refer to the Omics Pouch Instructions for Use (29492826).
2. Gently pass the cell suspension over the 100 µm cell strainer.
3. Transfer 5 mL of 10% FBS in DPBS into the Omics Pouch using a Luer-Lok syringe.
4. Gently massage the pouch to loosen any cells and rinse the pouch.
5. Extract the contents of the pouch into the same Luer-Lok syringe.
6. Gently pass the cell suspension over the 100 µm cell strainer.
7. Remove the cell strainer, cap the centrifuge tubes, and centrifuge the cell suspension at 300 × g for 10 minutes at 4°C.
8. Place the samples back onto ice.
9. Remove the supernatant with a 10 mL serological pipette.
10. Add 1 mL of DPBS/BSA/EDTA solution to the cell pellet.
11. Resuspend the cell pellet using a 1 mL pipette and 1000 µL wide-bore pipette tip. Make sure that the sample is fully resuspended.
12. Add 14 mL of 1× RBC lysis solution to each sample and invert up to 5 times to mix.
13. Incubate sample on ice for 5 minutes.
14. Centrifuge the cell suspension at 300 × g for 10 minutes at 4°C.
15. For single cell preparation only: Prepare two 50 mL centrifuge tubes; one with a 70 µm cell strainer and the other with a 40 µm cell strainer.
16. For single cell preparation only: Add 1 mL DPBS/BSA/EDTA solution to the cell strainers.
17. Remove the samples from the centrifuge and place back on ice.
18. Remove the supernatant by pouring or by using a 25 mL serological pipette.
19. Resuspend the cell pellet in 3 mL DPBS/BSA/EDTA solution using a 5 mL serological pipette.
20. For single cell preparation only: Pour the cell suspension onto the 70 µm cell strainer. Wait for the cells to pass through the strainer. Gently agitate the strainer if necessary.
21. For single cell preparation only: Repeat step 20 with the 40 µm cell strainer.
22. Proceed to cell counting and single cell workflow.

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