Procedure

Semi-automated enzymatic digestion using the dissociation enzyme mix A (lung), B (liver), C (brain), or D (kidney)

Introduction

This procedure provides recommendations for performing enzymatic dissociation in single-cell workflows using the omics bundle (VIA Extractor™* tissue disaggregator, VIA Freeze™ Uno controlled-rate freezer, and Omics clamp) and Omics pouch at 4°C for lung and brain tissue; 37°C for liver and kidney tissue. For manual tissue dissociation, please refer to the manual enzymatic digestion procedure in the related documents section here.

Required materials

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

Source	Material
Provided in the dissociation enzyme mix A	• Enzyme 3 • Enzyme 5 • Enzyme 6
Provided in the dissociation enzyme mix B	• Enzyme 3 • Enzyme 5
Provided in the dissociation enzyme mix C	• Enzyme 4 • Enzyme 5
Provided in the dissociation enzyme mix D	• Enzyme 1 • Enzyme 2
Equipment provided by the user	• Omics bundle (VIA Extractor tissue disaggregator, VIA Freeze Uno controlled-rate freezer, and Omics clamp) • Omics pouch • Centrifuge with refrigeration • Heat sealer • Micro pipettes • Pipette controller
Materials provided by the user for lung tissue dissociation	• Mouse lung tissue sample: 110–150 mg cut into 5 mm pieces • 50 mL conical centrifuge tubes • 5 mL syringes with locking luer connector • Cell strainer for 50 mL centrifuge tubes: 70 µm • 1000 µL wide-bore pipette tips • 25 mL, 10 mL, and 5 mL serological pipettes • Red blood cell removal/lysis kit (available from a third party) • Water for cell culture • Dulbecco's phosphate-buffered saline (DPBS) • Micropipette tips • Bovine serum albumin (BSA) • Refrigerated fetal bovine serum (FBS) • 25 mL reservoir • 0.2 µm sterile filter

Source	Material
Materials provided by the user for liver tissue dissociation	• Mouse liver tissue sample: 700 mg to 1000 mg with gall bladder removed • 50 mL conical centrifuge tubes • 5 mL syringes with locking luer connector • Cell strainer for 50 mL centrifuge tubes: 70 µm • 1000 µL wide-bore pipette tips • 25 mL, 10 mL, and 5 mL serological pipettes • Red blood cell removal/lysis kit (available from a third party) • Water for cell culture • Dulbecco's modified eagle medium (DMEM) supplemented with 4 mM L-glutamine • Dulbecco's phosphate-buffered saline (DPBS) • Micropipette tips • Bovine serum albumin (BSA) • Fetal bovine serum (FBS) • 25 mL reservoir • Debris removal solution (available from a third party) • 0.2 µm sterile filter
Materials provided by the user for brain tissue dissociation	• Adult mouse brain tissue sample up to 500 mg • 50 mL conical centrifuge tubes • 5 mL syringes with locking luer connector • Cell strainer for 50 mL centrifuge tubes: 70 µm • 1000 µL wide-bore pipette tips • 25 mL, 10 mL, and 5 mL serological pipettes • Red blood cell removal/lysis kit (available from a third party) • 10× Dulbecco's phosphate-buffered saline (DPBS) • Cytiva's Percoll™ solution • Water for cell culture • Hank's Balanced Salt Solution (HBSS) • Micropipette tips • Bovine serum albumin (BSA) • Refrigerated fetal bovine serum (FBS) • 25 mL reservoir • 0.2 µm sterile filter

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Source	Material
Materials provided by the user for kidney tissue dissociation	• Mouse kidney tissue sample: up to 300 mg • 50 mL conical centrifuge tubes • 5 mL syringes with locking luer connector • Cell strainer for 50 mL centrifuge tubes: 100 µm, 70 µm, and 40 µm • 1000 µL wide-bore pipette tips • 25 mL, 10 mL, and 5 mL serological pipettes • Red blood cell removal/lysis kit (available from a third party) • Water for cell culture • Dulbecco's modified eagle medium (DMEM) supplemented with 4 mM L-glutamine • Hank's Balanced Salt Solution (HBSS) • Dulbecco's phosphate-buffered saline (DPBS) • Micropipette tips • Bovine serum albumin (BSA) • EDTA (ethylenediaminetetraacetic acid) • Fetal bovine serum (FBS) • 25 mL reservoir • 0.2 µm sterile filter

Preparation

Note:

- For cold tissue dissociation, keep all reagents and tissue on ice, prechill the centrifuge to 4°C, and carry out benchwork on ice.
- When following the warm tissue dissociation protocol, keep all reagents and tissue at room temperature (RT) and perform all benchmarks at RT.
- Tissue should be stored in the storage solution. See the previous section for details on which storage solution is required for each tissue type.

1. Prepare the quench solution and the resuspension solution according to the table below.

Kit-Tissue	Quench solution	Resuspension solution	Storage solution
Kit A-Lung	DPBS+10% (v/v) FBS	DPBS+0.5% (w/v) BSA	DPBS
Kit B-Liver	DMEM+10% (v/v) FBS	DPBS+0.5% (w/v) BSA	DMEM
Kit C-Brain	DPBS+10% (v/v) FBS	DPBS+0.5% (w/v) BSA	HBSS
Kit D-Kidney	DPBS+10% (v/v) FBS	DPBS+0.5% (w/v) BSA +0.1 M EDTA	DMEM

2. Prepare the Dissociation enzyme mix A, B, C, or D as required:

   a. Reconstitute 1 vial of enzyme according to the table below.

	Mix A - lung	Mix B - liver	Mix C - brain
Reagent	Volume for 1 Vial	Volume for 1 Vial	Volume for 1 Vial
Enzyme 3	18.3 mL	18.3 mL	-
Enzyme 4	-	-	14 mL
Enzyme 5	8.06 mL	8.06 mL	8.06 mL
Enzyme 6	4.25 mL	-	-

Note:

- All enzymes resuspend in **DPBS.**
- **Enzymes 4 and 6**—Incubate the resuspended powder at 30°C to completely dissolve the enzyme.
- All enzymes must filter sterilize by passing it through a 0.22 µm filter.
- **Enzyme 1 and 2** in Enzyme kit D supplied reconstituted/ready to use.

   b. Prepare the enzyme mixture according to the table below.

	Mix A - lung	Mix B - liver	Mix C – brain	Mix D - kidney
Reagent	Volume for 1 sample	Volume for 1 sample	Volume for 1 sample	Volume for 1 sample
Enzyme 1	-	-	-	0.5 µL
Enzyme 2	-	-	-	125 µL
Enzyme 3	1 mL	1 mL	-	-
Enzyme 4	-	-	1 mL	-
Enzyme 5	10 µL	10 µL	10 µL	-
Enzyme 6	160 µL	-	-	-
Buffer volume	3.84 mL	3.99 mL	3.99 mL	4.875 mL
Total volume	5 mL	5 mL	5 mL	5 mL

Note:

- To calculate volumes for multiple samples, multiply the volume for one sample by the number of samples and add 3%.
- For cold tissue dissociation, keep enzyme mixes on ice. When following the warm tissue dissociation protocol, warm the enzyme mix at 37°C for 30 minutes.
- Use DBPS as the buffer for Kit A, B, and C.
- Use HBSS as the buffer for Kit D.

Dissociation protocol

Follow the steps below to efficiently dissociate the tissue sample using the VIA Extractor™ tissue disaggregator. With the exception of the liver and kidney tissue, keep the samples on ice and the reagents cold during the protocol to minimize 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.
   - VIA Extractor tissue disaggregator operating in standard mode, with speed and time parameters set according to the table below.

Kit-Tissue	VIA Extractor tissue disaggregator speed	VIA Extractor tissue disaggregator time	Temperature setting
Kit A-Lung	200 rpm	60 min	2°C
Kit B-Liver	60 rpm	22 min	37°C
Kit C-Brain	200 rpm	30 min	2°C
Kit D-Kidney	200 rpm	10 min	37°C

2. Wait for the VIA Extractor tissue disaggregator to reach the set temperature.

   Note: Ensure the temperature of the VIA Extractor tissue disaggregator is correct before starting the dissociation protocol. The program temperature is set at 2°C to allow the VIA Extractor tissue disaggregator to dissociate at 4°C.

3. Add one tissue sample to the omics pouch and heat seal the omics pouch. Refer to the *Omics Pouch Instructions for Use (29492826)* and the heat seal process for the Omics Pouch for more information.
4. Using a locking Luer syringe, add 5 mL of enzyme mixture as the digestive solution into each compartment of the omics pouch. Refer to the *Omics Pouch Instructions for Use (29492826)* for more information.
5. Run the protocol on the VIA Extractor tissue disaggregator.

Prepare for post-dissociation processing

During the protocol run on the VIA Extractor tissue disaggregator, follow the steps below to prepare for the post-dissociation processing.

1. Place one 100 µm cell strainer for kidney tissue or a 70 µm cell strainer for lung, brain and liver on top of one 50 mL conical centrifuge per sample.
2. Wet each cell strainer with 2 mL of quench solution.

Sample straining

Once the dissociation run on the VIA Extractor tissue disaggregator is complete, follow the steps below to perform sample straining.

Note: To minimize cellular aggregation, perform the post-dissociation steps swiftly. For liver and kidney tissue, keep reagents and samples at RT; for all other tissues, keep reagents and samples on ice.

1. Remove the omics pouch from the omics clamp.
2. Extract the cell suspension from the omics pouch through the luer lock, for further details, refer to the *Omics Pouch Instructions for Use (29492826)*.
3. Gently pass the cell suspension over the cell strainer.
4. Add 5 mL of quench solution into the omics pouch using a locking luer syringe.
5. Gently massage the pouch to loosen any cells.
6. Extract the contents of the pouch into the same locking luer syringe.
7. Gently pass the cell suspension over the cell strainer.
8. Rinse the cell strainer with 2 mL of quench solution.
9. Remove the cell strainer, cap the centrifuge tubes, and centrifuge the cell suspension at 300 × g for 10 minutes.
10. Place the samples back onto the ice. For liver and kidney tissue store at room temperature.
11. Remove the supernatant and resuspend the pellet in resuspension buffer.
12. For optimal results, additional steps including red blood cell (RBC) removal or debris removal might be required. For brain tissue post processing, refer to 'Myelin removal for brain tissue'.

Post-processing for kidney tissue

For kidney tissue, the following steps might be required to remove cell clumps. Perform the following steps on ice after RBC removal step.

1. Add 3 mL of resuspension solution to cell pellet after red blood cell removal.
2. Mix thoroughly by pipetting with a 5 mL serological pipette to resuspend the pellet.
3. Place a 70 µm and a 40 µm strainer on separate 50 mL centrifuge tubes.
4. Wet each strainer with 1 mL of resuspension solution.
5. Pass the sample over the 70 µm cell strainer and wait until the sample has passed through.
6. Pass the sample over the 40 µm cell strainer and wait until the sample has passed through.

Post-processing for liver tissue

For optimal results, it is recommended to perform RBC removal followed by debris removal.

Post-processing for lung tissue

For optimal results, it is recommended to perform RBC removal.

Myelin removal for brain tissue

1. Perform RBC removal as per the manufacturer's protocol.
2. Dilute 2 mL of 10× DPBS in 18 mL of Percoll solution. Keep on ice.
3. Add 15 mL of the above solution of DPBS and Percoll solution to 35 mL of ice-cold 1× DPBS to make a 27% Percoll solution. Keep on ice.
4. Resuspend the cell pellet after RCB removal in 1mL DPBS + 0.5% BSA. Pipette mix gently with a 1 mL wide-bore tip.
5. Measure dissociated brain cell suspension using a 2 mL serological pipette, note the volume of the cell pellet, and transfer the pellet to a 15 mL centrifuge tube.
6. Add 6 mL of 27% Percoll solution per 1mL of brain sample. Mix gently by inverting the tube.
7. Centrifuge at 4°C at 700 × g for 10 minutes with the brake off (change deceleration to 0 on the centrifuge).
8. Carefully remove and discard the upper myelin layer using a 2 mL serological pipette.
9. Remove and discard the remaining supernatant using a serological pipette.
10. Resuspend cells in 5 mL resuspension solutionand slowly pipette the pellet to wash.
11. Change centrifuge deceleration settings to maximum and centrifuge at 300 × g for 10 minutes.
12. Remove the supernatant and resuspend the pellet in 1 mL resuspension solution.

Troubleshooting for brain tissue

Given the stringy nature of cells in brain tissue, it is normal for the cell strainer to clog. If this happens after brain tissue dissociation, follow the steps below.

1. Wet a 70 µm cell strainer with 2 mL quench solution.
2. Pass 2.5 mL of digested material through the cell strainer into a 50 mL conical centrifuge tube on ice.
3. Add 2 mL quench solution to help the digested material pass through the cell strainer.
4. Gently push the tissue material remaining in the cell strainer through the cell strainer using the plunger from a 5 mL syringe (or similar).
5. Wash the cell strainer by passing 1 mL quench solution over the strainer 3 times.
6. Repeat steps 2–5 until all of the digested material is strained.

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