Procedure

Manual 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 manual tissue dissociation at 4°C or 37°C. For automated tissue dissociation with the Omics bundle (VIA Extractor™* tissue disaggregator, VIA Freeze™ Uno controlled-rate freezer, omics clamp) and omics pouch, please refer to the semi-automated 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	• Micropipettes • Thermo-mixer or heating device that can heat to 37°C • Pipette controller • Centrifuge with refrigeration
Materials provided by the user for lung tissue dissociation	• Mouse lung tissue sample: 110–150 mg cut into 5 mm pieces • 50 mL sterile conical centrifuge tubes • 15 mL centrifuge tube • 70 µm cell strainer • 40 µm cell strainer • 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) • Fetal bovine serum (FBS) • Sterile 25 mL reservoir • 0.2 µm sterile filter

*For research use only.

Source	Material
Materials provided by the user for liver tissue dissociation	• Mouse liver tissue sample: 400–500 mg with gall bladder removed • 50 mL sterile conical centrifuge tubes • 15 mL centrifuge tube • 70 µm cell strainer • 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 high glucose • Dulbecco's phosphate-buffered saline (DPBS) • Micropipette tips • Bovine serum albumin (BSA) • Refrigerated fetal bovine serum (FBS) • Sterile 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 samples up to 500 mg • 50 mL conical centrifuge tubes • 15 mL centrifuge tube • 70 µm cell strainer • 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 • Hank's balanced salt solution (HBSS) • 10× Phosphate-buffered saline (PBS) • Cytiva's Percoll™ solution • Dulbecco's phosphate-buffered saline (DPBS) • Micropipette tips • Bovine serum albumin (BSA) • Fetal bovine serum (FBS) • 25 mL reservoir • 0.2 µm sterile filter

Source	Material
Materials provided by the user for kidney tissue dissociation	• Mouse kidney tissue sample: up to 300mg • 50 mL conical centrifuge tubes • 100 µm cell strainer • 70 µm cell strainer • 40 µm cell strainer • 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 high glucose • 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 as detailed in 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 +0.1M 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	-	-	-	1 µL
Enzyme 2	-	-	-	250 µL
Enzyme 3	2 mL	1 mL	-	-
Enzyme 4	-	-	2 mL	-
Enzyme 5	20 µL	10 µL	20 µL	-
Enzyme 6	300 µL	-	-	-
DMEM	-	2990 µL	-	-
HBSS	-	-	-	4.749 mL
Total volume	2.32 mL	4 mL	2.02 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.
- Recommended sample sizes, buffers, dissociation temperatures, and time for manual dissociation are provided in the table below.

Mix and tissue type	Size/weight	Storage solution	Quench solution	Resuspension buffer	Cell strainer size	Temperature (°C)	Time
Mix A – lung	Mouse lungs, 110 to 160 mg	DPBS	DPBS + 10% Fetal bovine serum (FBS)	DPBS + 0.5% BSA	70 µm and 40 µm	37	30 min
Mix B – liver	Liver tissue, 400 to 600 mg	DMEM	DMEM + 10% FBS	DPBS + 0.5% BSA	70 µm	37	30 min
Mix C – brain	1 adult brain up to 600 mg	HBSS	DPBS + 10% FBS	DPBS + 0.5% BSA	70 µm	4 and 37	30 min
Mix D – kidney	1 kidney up to 500 mg	DMEM	DPBS + 10% FBS	DPBS + 0.5% (w/v) BSA + 0.1M EDTA	100 µm, 70 µm, and 40 µm	37	15 min

Manual dissociation protocol

1. Mince the tissue by cutting finely with a scalpel.
2. Transfer the minced tissue to a 15 mL centrifuge tube.
3. Add the enzyme mix to the tissue.
4. Triturate the sample 10 times with a wide-bore 1 mL pipette (T=0, 10, 20, and 30 min).
5. Incubate for 15 or 30 minutes, repeating step 4 every 5 (T=0, 5, 10, and 15 min) or 10 minutes (T=0, 10, 20, and 30 min).
   - For warm dissociation: Incubate on a dry bath/block incubator at 37°C.
   - For cold dissociation: Incubate on ice.
6. 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. Wet the cell strainer with 2 mL of quench solution.
7. Pass the digested material through the cell strainer into a 50 mL conical centrifuge tube.
8. Rinse the digestion vessel with 2 mL of quench solution and pass the resultant solution over the strainer.
9. Use the plunger from a 5 mL syringe (or similar) to gently push the tissue material remaining in the filter through the filter.
10. Wash filter 3 times, with 1 mL quench solution.
11. Centrifuge the samples at 300 x g for 10 minutes.
12. Remove the supernatant and resuspend the pellet in resuspension solution.
13. For optimal results, additional steps, including red blood cell (RBC) removal or debris removal might be required. For brain post processing, refer to ‘Myelin removal for brain tissue’.

Post-processing for lung tissue

1. For dissociation of lung tissue, the following steps are optional to remove cell clumps. Perform the following steps at RT after the RBC removal step. Add 1 mL of resuspension solution to the cell pellet after red blood cell lysis.
2. Mix thoroughly by pipetting with a 5 mL serological pipette to resuspend the pellet.
3. Place a 40 µm strainer on separate 50 mL centrifuge tubes.
4. Pass the sample over the 40 µm cell strainer and wait until the sample has passed through.

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 RBC removal.
2. Mix pellet thoroughly in resuspension solution with 5 mL serological pipette.
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 red blood cell removal followed by debris 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 the mixture 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 27% Percoll solution. Keep on ice.
4. Resuspend the cell pellet after RCB removal in 1 mL 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 1 mL of brain sample. Mix gently by inverting the tube.
7. Centrifuge at 4°C at 700 x 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 DPBS + 0.5% BSA and 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 DPBS + 0.5% BSA.

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