Procedure

# Manual enzymatic digestion using the dissociation enzyme mix A (lung), B (liver) or C (brain)

## 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<br>• Enzyme 5<br>• Enzyme 6 |
| **Provided in the dissociation enzyme mix B** | • Enzyme 3<br>• Enzyme 5 |
| **Provided in the dissociation enzyme mix C** | • Enzyme 4<br>• Enzyme 5 |
| **Equipment provided by the user** | • Micropipettes<br>• Thermo-mixer or heating device that can heat to 37°C<br>• Pipette controller |
| **Consumables provided by the user for the lung** | • Mouse lung tissue sample: 110-150 mg cut into 5 mm pieces<br>• 50 mL sterile conical centrifuge tubes<br>• 15 mL centrifuge tube as a digestion vessel<br>• Cell strainer for 50 mL centrifuge tubes: 70 µm and 40 µm<br>• 1000 µL wide-bore pipette tips<br>• 25 mL, 10 mL, and 5 mL serological pipettes<br>• Red blood cell lysis solution<br>• Water for cell culture<br>• Storage solution for tissue – Refrigerated Dulbecco's phosphate-buffered saline (DPBS)<br>• Dulbecco's phosphate-buffered saline (DPBS)<br>• Micropipette tips<br>• Bovine serum albumin (BSA)<br>• Fetal bovine serum (FBS)<br>• Sterile 25 mL reservoir<br>• Scalpel |

*For research use only.

| Source | Material |
|---|---|
| **Consumables provided by the user for the liver** | • Mouse liver tissue sample: 400- 500 mg with gall bladder removed<br>• 50 mL sterile conical centrifuge tubes<br>• 15 mL centrifuge tube<br>• Cell strainer for 50 mL centrifuge tubes: 70 µm<br>• 1000 µL wide-bore pipette tips<br>• 25 mL, 10 mL, and 5 mL serological pipettes<br>• Red blood cell lysis solution<br>• Water for cell culture<br>• Storage solution for tissue: Dulbecco's modified eagle medium (DMEM) supplemented with 4 mM L- glutamine<br>• Dulbecco's phosphate-buffered saline (DPBS)<br>• Dulbecco's minimum eagle medium (DMEM) supplemented with 4 mM L-glutamine<br>• Micropipette tips<br>• Bovine serum albumin (BSA)<br>• Refrigerated fetal bovine serum (FBS)<br>• Sterile 25 mL reservoir<br>• Scalpel<br>• Debris removal solution (available from a third party) |
| **Consumables provided by the user for the brain** | • Adult mouse brain tissue samples up to 500 mg<br>• 50 mL sterile conical centrifuge tubes<br>• 15 mL centrifuge tube as a digestion vessel<br>• Cell strainer for 50 mL centrifuge tubes: 70 µm<br>• 1000 µL wide-bore pipette tips<br>• 25 mL, 10 mL, and 5 mL serological pipettes<br>• Red blood cell lysis solution<br>• Water for cell culture<br>• Storage solution for tissue: Hank's Balanced Salt Solution (HBSS)<br>• Dulbecco's phosphate-buffered saline (DPBS)<br>• Micropipette tips<br>• Bovine serum albumin (BSA)<br>• Fetal bovine serum (FBS)<br>• Sterile 25 mL reservoir<br>• Scalpel<br>• For myelin removal Cytiva's Percoll™ solution for myelin removal |

## 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 |
|---|---|---|
| Kit A-Lung | DPBS+10% (v/v) FBS | DPBS+0.5% (w/v) BSA |
| Kit B-Liver | DMEM +10% (v/v) FBS | DPBS+0.5% (w/v) BSA |
| Kit C-Brain | DPBS+10% (v/v) FBS | DPBS+0.5% (w/v) BSA |

2. Prepare the dissociation enzyme mix A, B, or C, as required:
   a. Resuspend the enzymes, if applicable, according to the *Instructions for Use* for the specific enzyme mix, found here.
   b. Prepare the enzyme mixture according to the table below:

| | Mix A - lung | Mix B - liver | Mix C – brain |
|---|---|---|---|
| Reagent | Volume for 1 sample | Volume for 1 sample | Volume for 1 sample |
| 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 | - |
| Total volume | 2.32 mL | 4 mL | 2.02 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 | DPBS + 10% FBS | DPBS + 0.5% BSA | 70 µm | 37 | 30 min |
| Mix C - brain | 1 adult brain up to 600 mg | DPBS | DPBS + 10% FBS | DPBS + 0.5% BSA | 70 µm | 37 | 30 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 30 minutes, repeating step 4 every 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. Wet the appropriately sized cell strainer with 2 mL of quench solution.
7. Pass the digested material through the cell strainer into a 50 mL conical centrifuge.
8. Rinse the digestion vessel with 2 mL of quench solution and pass the resultant solution over the strainer (please refer to the section, materials provided by user for tissue specific recommendation on strainer size).
9. Use the plunger from a 5 mL syringe (or similar) to gently push the tissue material remaining in the filter through the filter (wash filter 3 times, with 1 mL DPBS/10% FBS).
10. Centrifuge the samples at 300 x g for 10 minutes.
11. Remove the supernatant and resuspend the pellet in resuspension solution.
12. For optimal results, additional steps, including red blood cell (RBC) removal, debris removal, or myelin removal, might be required.

## 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 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 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 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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