Brain Tissue Dissociation for Cell Sorting

Grant Lin,Michelle Monje

Abstract

This is our standard brain tissue dissociation protocol for cell sorting (Human Cell Atlas).

Citation: Grant Lin,Michelle Monje Brain Tissue Dissociation for Cell Sorting. **protocols.io** dx.doi.org/10.17504/protocols.io.nx7dfrn
Published: 27 Mar 2018

Guidelines

NOTE: A critical component of this protocol requires proper handling of the tissue donation starting immediately at the time of autopsy. The overall sample viability depends significantly on minimizing the post-mortem interval (PMI), immediately transferring tissue into the appropriate cold medium supplemented with antibiotics/antimycotics, keeping the sample on **wet** ice throughout transportation, and maintaining sterile technique throughout the protocol.

The protocols workflow is as follows:

1. Obtaining tissue samples (Steps 1-9)

2. Preparation for sample processing (Steps 10-15)

3. Mechanical dissociation (Steps 16-21)

4. Enzymatic dissociation (Steps 22-32)

5. Sucrose gradient centrifugation (Steps 33-39)

6. ACK red blood cell lysis (Steps 40-44)

Thumbnail Image:

https://www.humancellatlas.org/areas-of-impact

Materials

- Shipping box with an insulated container that can be directly used for return transportation of the tissue sample by Contributed by users
- Sterile gloves, drapes, and scalpels by Contributed by users
- 8-10 sterile 50 mL conical tubes by Contributed by users
- Shipping media by Contributed by users
- Sucrose by Contributed by users
- Distilled water by Contributed by users
- 10X Hank's Buffered Saline Solution (HBSS) without calcium or magnesium by Contributed by users
- HBSS with calcium and magnesium by Contributed by users
- Deoxyribonuclease I by Contributed by users
- Worthington STEMxyme I by Contributed by users
- 1M HEPES buffer by Contributed by users
- High-walled 100 x 20 mm cell culture dish by Contributed by users
- Culture media (e.g., Hibernate-A) by Contributed by users
- Razor blade by Contributed by users
- HBSS without calcium and magnesium by Contributed by users

Protocol

Obtaining tissue samples

Step 1.

Upon notification of an imminent donation, prepare a sample collection kit. Using a shipping box with an insulated container that can be directly used for return transportation of the tissue sample, include materials listed in the following steps.

NOTES

Grant Lin 21 Mar 2018

A critical component of this protocol requires proper handling of the tissue donation starting immediately at the time of autopsy. The overall sample viability depends significantly on minimizing the post-mortem interval (PMI), immediately transferring tissue into the appropriate cold medium supplemented with antibiotics/antimycotics, keeping the sample on **wet** ice throughout transportation, and maintaining sterile technique throughout the protocol.

Obtaining tissue samples

Step 2.

Include materials for sterile preparation, including sterile gloves, drapes, and scalpels.

Obtaining tissue samples

Step 3.

Include 8-10 sterile 50 mL conical tubes containing 30 mL shipping media in the insulated container with ice or cold packs.

AMOUNT

30 ml Additional info: Shipping media

NOTES

Grant Lin 21 Mar 2018

While any standard cell culture media can work, the best sample viability is obtained with Hibernate-A supplemented with antibiotic/antimycotic.

Obtaining tissue samples

Step 4.

Include any further sample tubes for other analyses (e.g. fixatives, RNA-later).

Obtaining tissue samples

Step 5.

If the autopsy will not be performed at the investigator's site, include a document that clearly states how to collect the tumor sample.

NOTES

Grant Lin 21 Mar 2018

This includes details such as maintaining sterility, keeping sample tubes on wet ice, and including samples from the midbrain and medulla as tumor cells often invade these regions.

Obtaining tissue samples

Step 6.

Maintain sterility during the autopsy. Consider the brain sterile inside the cranium, so observe sterile technique (including changing gloves) once the cranium is open. Avoiding contamination with skin and hair is critical.

Obtaining tissue samples

Step 7.

Dissect the brain region of interest. With a sterile scalpel, cut small 1 cm chunks from the tumor and immediately transfer into cold shipping media (see NOTE in step 3) and put on wet ice. Place 10 mL of tissue into each tube, resulting in a final volume of tissue and media in each tube of 40 mL.

Obtaining tissue samples

Step 8.

After sample collection, ship the samples overnight on **wet** ice in the insulated container.

NOTES
Grant Lin 21 Mar 2018

Do not ship the sample on dry ice, as freezing will kill the cells.

Preparation for sample processing
Step 9.

Prior to beginning sample preparation, sterilize tissue culture hoods along with razor blades, curved hemostats, and any other non-sterile tools under UV light for 1 hour.

NOTES
Grant Lin 21 Mar 2018

Perform all of the following steps under sterile conditions to prevent contamination of the cultures.

Preparation for sample processing
Step 10.

Prepare solutions as follows.

[Sucrose Solution] To prepare 1.8 M sucrose solution, first, dissolve 308.07 g sucrose in 300 mL distilled water.

AMOUNT
308.07 g Additional info: Sucrose
AMOUNT
300 ml Additional info: Distilled water
Preparation for sample processing
Step 11.

[Sucrose Solution] Add 50 mL 10X Hank's Buffered Saline Solution (HBSS) **without** calcium or magnesium and bring total volume to 500 mL using distilled water. Store at 4°C.

AMOUNT
50 ml Additional info: 10X Hank's Buffered Saline Solution (HBSS)
TEMPERATURE
4 °C Additional info: Storage
Preparation for sample processing
Step 12.

[**Enzymatic Digestion Solution**] To prepare enzymatic digestion solution, first, take 50 mL HBSS **with** calcium and magnesium for every 10 mL of minced tissue and add 500 µL 5 mg/mL deoxyribonuclease I (final concentration 50 µg/mL), 500 µL 25 mg/mL Worthington STEMxyme I (final concentration 250 µg/mL), and 500 µL 1M HEPES buffer.

AMOUNT

50 ml Additional info: HBSS with calcium and magnesium for every 10 mL

AMOUNT

500 µl Additional info: 5 mg/mL deoxyribonuclease I

AMOUNT

500 µl Additional info: 25 mg/mL Worthington STEMxyme I

AMOUNT

500 µl Additional info: 1M HEPES buffer

NOTES

Grant Lin 21 Mar 2018

Different enzymatic digestions solution mixes can be used at this step. We have also used Liberase DH at a final concentration of 25 µg/mL.

Preparation for sample processing

Step 13.

Filter the Sucrose and Enzymatic Digestion solutions through a sterile (0.22µm) filter

Preparation for sample processing

Step 14.

[**Enzymatic Digestion Solution**] Prewarm digestion solution in a 37 °C water bath.

TEMPERATURE

37 °C Additional info: Digestion solution

Preparation for sample processing

Step 15.

Pre-cool a laboratory centrifuge equipped with a swinging-bucket rotor to 4°C.

TEMPERATURE

4 °C Additional info: Pre-cooling centrifuge

Mechanical dissociation

Step 16.

Transfer the tissue into a high-walled 100 x 20 mm cell culture dish.

Mechanical dissociation

Step 17.

Remove media leftover from shipping and replace with 10-15 mL cold culture media (e.g., Hibernate-A).

Mechanical dissociation

Step 18.

Using the curved hemostats to grasp a razor blade, mince the tissue finely while removing obvious blood vessels or meninges.

NOTES

Grant Lin 21 Mar 2018

The final tissue fragments should be smaller than 1 mm.

Mechanical dissociation

Step 19.

Transfer the tissue into a clean 50 mL conical tube.

Mechanical dissociation

Step 20.

Wash the cell culture dish with an additional 5 mL cold culture media and transfer to the conical tube. Repeat this wash step as necessary to transfer remaining tissue.

AMOUNT

5 ml Additional info: Cold culture media

Mechanical dissociation

Step 21.

Using a 10 mL serological pipet, triturate gently (4-5 times). Allow larger tissue fragments to settle to the bottom of the tube.

Enzymatic dissociation

Step 22.

If there is more than 5 mL of tissue in the tube containing the minced tissue fragments, split the tissue into other new 50 mL conical tubes such that no tube has more than 5 mL tissue.

Enzymatic dissociation

Step 23.

Centrifuge the conical tube(s) containing the remaining tissue fragments for 5 min (350 x g, 4°C).

TEMPERATURE

4 °C Additional info: Centrifugation

Enzymatic dissociation

Step 24.

Remove the supernatant and add the prewarmed enzymatic digestion solution, such that there is 5 mL digestion solution for every 1 mL tissue.

NOTES

Grant Lin 21 Mar 2018

E.g. 25 mL digestion solution for 5 mL tissue.

Enzymatic dissociation

Step 25.

Seal the conical tube lids with laboratory film and incubate the reaction on a rotator at 37°C for 30 min.

TEMPERATURE
37 °C Additional info: Incubation

Enzymatic dissociation
Step 26.

After the incubation, to triturate the samples gently, first, using a 10 mL serological pipet, pipet the sample up and down 6-8 times.

NOTES
Grant Lin 21 Mar 2018

Avoid generating excessive air bubbles.

Enzymatic dissociation
Step 27.

Add a 1000 µL pipet tip to the end of the pipet and triturate an additional 6-8 times.

Enzymatic dissociation
Step 28.

Allow any remaining chunks to settle to the bottom of the tube.

Enzymatic dissociation
Step 29.

Remove and filter the supernatant with the cells still suspended through a 100 µm filter into a new 50 mL conical tube labeled 'Enzymatic Dissociation' and store on ice.

Enzymatic dissociation
Step 30.

If significant tissue fragments remain in the unfiltered fraction, add an additional 10 mL HBSS with calcium and magnesium to the fragments and repeat steps 25-28, combining the additional HBSS into the previously filtered supernatant in the 'Enzymatic Dissociation' tube.

GOTO
Repeating steps 26 through 29 -> go to step #26

Enzymatic dissociation
Step 31.

Centrifuge the 'Enzymatic Dissociation' tube for 5 min (350 x g, 4°C) and continue to sucrose gradient centrifugation (next step).

TEMPERATURE
4 °C Additional info: Centrifugation

Sucrose gradient centrifugation

Step 32.

If samples are still suspended in solution, centrifuge for 5 min (350 x g, 4°C)

TEMPERATURE
4 °C Additional info: Centrifugation

Sucrose gradient centrifugation

Step 33.

Remove the supernatant and resuspend tissue in 20 mL cold HBSS without calcium and magnesium. Bring volume up to 25 mL total with cold HBSS.

AMOUNT
20 ml Additional info: Cold HBSS without calcium and magnesium

Sucrose gradient centrifugation

Step 34.

Slowly add 25 mL 1.8 M sucrose solution and invert the tube to mix.

AMOUNT
25 ml Additional info: 1.8 M sucrose solution

NOTES
Grant Lin 21 Mar 2018

This results in a 0.9 M sucrose gradient.

Sucrose gradient centrifugation

Step 35.

Centrifuge **with no brake** for 10 min (800 x g, 4°C).

TEMPERATURE
4 °C Additional info: Centrifugation

NOTES
Grant Lin 21 Mar 2018

Using a centrifugation brake will disrupt the gradient and reduce yield.

Sucrose gradient centrifugation

Step 36.

Carefully aspirate myelin debris and as much sucrose solution as possible.

Sucrose gradient centrifugation

Step 37.

Wash the sample by adding 30 mL cold HBSS without calcium and magnesium and mixing gently.

AMOUNT
30 ml Additional info: Cold HBSS without calcium and magnesium

Sucrose gradient centrifugation

Step 38.

Centrifuge for 5 min (350 x g, 4°C).

TEMPERATURE
4 °C Additional info: Centrifugation

ACK red blood cell lysis

Step 39.

Remove the wash supernatant.

ACK red blood cell lysis

Step 40.

Add 5 mL ACK lysis buffer and gently resuspend the cell pellet, swirling the tube for 1 min at room temperature.

AMOUNT
5 ml Additional info: ACK lysis buffer

ACK red blood cell lysis

Step 41.

Quench the lysis by adding 30 mL cold HBSS without calcium and magnesium.

AMOUNT
30 ml Additional info: Cold HBSS without calcium and magnesium

ACK red blood cell lysis

Step 42.

Centrifuge for 5 min (350 x g, 4°C).

TEMPERATURE
4 °C Additional info: Centrifugation

ACK red blood cell lysis

Step 43.

Resuspend samples in appropriate staining buffer for subsequent analysis, such as flow cytometry and fluorescence-activated cell sorting.

Warnings

For safety warnings and hazard information please refer to the SDS (Safety Data Sheet).