Mar 30, 2023

# Single cell dissociation of brain organoids

In 2 collections

DOI
dx.doi.org/10.17504/protocols.io.e6nvwj799lmk/v1

michela.deleidi[1], Maria Jose Perez J.[1], Hariam Raji[1]

[1]German Center for Neurodegenerative Diseases (DZNE), Tübingen, 72076 Germany

Federico Bertoli
ASAP





**DOI: https://dx.doi.org/10.17504/protocols.io.e6nvwj799lmk/v1**

**Protocol Citation:** michela.deleidi, Maria Jose Perez J., Hariam Raji 2023. Single cell dissociation of brain organoids. **protocols.io https://dx.doi.org/10.17504/protocols.io.e6nvwj799lmk/v1**



**Protocol status:** Working
**We use this protocol and it's working**

**Created:** March 28, 2023

**Last Modified:** May 31, 2024

**Protocol Integer ID:** 79538

**Keywords:** brain organoids, cell dissociation, Papain Dissociation System, ASAPCRN, single cell dissociation of brain organoid, brain organoid, single cell dissociation, protocol details about single cell dissociation, cell dissociation, cell, brain

## Abstract

This protocol details about single cell dissociation of brain organoids.

## Attachments

405-879.docx

15KB

## Materials

**Kit:**

Papain Dissociation System.

Papain Dissociation System **Worthington Biochemical Corporation** **Catalog #**LK003150

**Reconstitute powders**.

- Add 5 mL Earle´s medium into Papain Vial (1 Vial/2 organoids).
- Add 500 µL Earles´s medium into DNAse vial.
- Add 35 mL Earle´s medium into Inhibitor vial (1 vial/10 organoids).

## Troubleshooting

## Single cell dissociation of brain organoids

27m

1 Mix 500 µL DNAse with 5 mL Papain.

Note

**Note**: MIX GENTLY.

2 Transfer single or pooled organoid to 60 mm dish.

3 Aspirate excess media, add 2.5 mL Papain + DNAse solution.

4 With a razor blade mince organoid (<1 mm).

5 Transfer plate to an orbital shaker 70 rpm, 00:30:00 (inside incubator).

6 With 1-mL pipette dissociate pieces (Mix up-down 30 times).

7 Put in orbital shaker 00:20:00 .

20m

8 In the meantime, add 5 mL Earle´s medium + 3 mL Inhibitor to a 15-mL conical tube.

9 Remove samples from the orbital shaker. With a 1-mL tip, mix up-down 30 times.

10 Take 2 mL (upper part) into new tube using a 40 µm cell strainer. Wait 1-3 min to debris to settle.

11 Transfer cell suspension to the inhibitor tube. Invert to mix 5 times.

12 Centrifuge 300 rpm, Room temperature, 00:07:00 . 7m

13 Aspirate supernatant, resuspend in 500 µL to 1 mL 0.5% BSA-PBS (Up-down 30 times).

14 Filter the resuspended cells ( 900 µL ) with a 30 µm cell strainer.

15 Count the cells for the final suspension and dilute. Resuspend at 1000 cells/µl in 0.04% BSA-PBS.