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We use this protocol and it's working

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Single cell dissociation of brain organoids

In 6 collections

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ABSTRACT

This protocol details about single cell dissociation of brain organoids.

ATTACHMENTS

405-879.docx

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).

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.