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 We use this protocol and it's working

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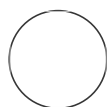
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# Single cell dissociation of brain organoids

In 1 collection

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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 Earle'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  $\mu$ L 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.