# Single cell dissociation of brain organoids

Kit: Papain Dissociation System. Worthington Biochemical. Catalog number: LK003150

Reconstitute powders.

* Add 5ml Earle´s medium into Papain Vial (1 Vial/2 organoids)
* Add 500µl Earles´s medium into DNAse vial
* Add 35mL Earle´s medium into Inhibitor vial (1 vial/10 organoids)

1. Mix 500µl DNAse with 5mL Papain (MIX GENTLY)
2. Transfer single or pooled organoid to 60mm dish
3. Aspirate excess media, add 2,5 mL Papain + DNAse solution
4. With a razor blade mince organoid (<1mm)
5. Transfer plate to an orbital shaker (70rpm inside incubator) 30 min.
6. With 1 mL pipette dissociate pieces (Mix up-down 30 times)
7. Put in orbital shaker 20 minutes.
8. In the meantime, add 5mL Earle´s medium + 3ml Inhibitor to a 15mL conical tube.
9. Remove samples from the orbital shaker. With a 1mL 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 300g 7 minutes, ROOM TEMPERATURE.
13. Aspirate supernatant, resuspend in 500µL-1mL 0,5% BSA-PBS (Up-down 30 times).
14. Filter the resuspended cells (900µL) with a 30 µm cell strainer.
15. Count the cells for the final suspension and dilute. Resuspend at 1000 cells/ul in 0,04% BSA-PBS