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Fixation, cryosectioning and immunostaining brain organoids

**Materials**

* 4%-5% PFA in PBS
* PBS
* 30% Sucrose in PB
* 2X PB: 6.37g NaH2PO4/H2O + 51.45g Na2HPO4/H2O + water to 1 Liter
* Gelatin/sucrose solution: 7.5% gelatin in 10% sucrose in PB
* Permeabilization/Blocking solution: 0.25% Triton-X, 4% normal donkey serum in PBS
* Blocking solution: 0.1% Triton-X, 4% normal donkey serum in PBS

Procedures

**Fixing tissues and sucrose sinking**

1. Remove media from floating tissues and replace with PFA. Fix at 4C for 15-20 min.
2. Wash 3X for 10 min in PBS.
3. Allow tissue to sink in 30% sucrose solution overnight.

**Embedding tissue in gelatin**

1. Warm gelatin/sucrose to 37C to liquefy before use.
2. Prepare weigh boats with a layer of gelatin/sucrose and place at 4C to harden.
3. Incubate tissue in gelatin/sucrose at 37C for ~15min.
4. Put tissue on top of hardened gelatin in weigh boat and drop a little gelatin next to tissue to hold it in place.
5. Then cover tissue with gelatin/sucrose and allow to solidify at 4C.
6. Cut out a block of gelatin with tissue inside being careful to cut straight for the side that will be oriented down, and cut out a corner to orient if necessary.
7. Use tissue-tek to glue gelatin/tissue block to a small piece of rough cardboard (be sure to glue to the rough side of cardboard piece).
8. Prepare cold isopentane bath by pouring isopentane into a beaker and adding dry ice until it reaches -35C to -50C (measure using a low temp thermometer).
9. Drop freeze gelatin/tissue block in isopentane bath ~1min for one tissue or a maximum of 2min for 3-4 tissues in a block.
10. Wrap frozen block in aluminum foil being careful to maintain cold temperature.
11. Place on dry ice temporarily and store frozen block at -80C.

**Sectioning**

1. Equilibrate tissue block to the temperature within cryostat for 10min. Cryostat should be set at -14 to -18C ambient temp/blade temp and -13 to -18C block temp.
2. Mount frozen block on metal disk by using tissue-tek to glue cardboard to frozen disk and cover cardboard with tissue-tek to ensure that it is well attached.
3. Trim block so that not too much of the section is embedding material.
4. Cut sections at anywhere from 10um to 60um and avoid rolling by using an anti-roll bar as well as a fine brush to unroll and position section for collection.
5. Collect section on Superfrost Plus slide keeping track of order of sections on the slides. Be careful that you do not get fingerprints on the surface of the slide as the oil in your fingers will inhibit attachment.
6. Allow sections to dry on slide for several hours or a maximum of overnight, then store at -80C.

**Immunostaining of cryosections**

1. Thaw slide with sections and allow to dry.
2. Use a pap-pen or liquid blocker pen to draw a border around the sections on the slide and allow to dry.
3. Rinse slide in PBS.
4. Block and permeabilize by adding at least 300ul of 0.25%TX blocking solution to area containing sections. Block for 1 hour at room temperature in a humidified chamber.
5. Stain with primary antibody in 300ul blocking solution per slide overnight in humidified chamber at room temperature.
6. Wash 3X PBS.
7. Stain with secondary antibody in blocking solution for 2 hours in humidified chamber at room temperature.
8. Wash 3X PBS.
9. Mount with a cover glass using Citifluor and seal using clear nail polish.