Video Article

Flash Freezing and Cryosectioning E12.5 Mouse Brain

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URL: http://www.jove.com/index/Details.stp?ID=198
DOI: 10.3791/198
Citation: Currle D.S., Monuki E.S. (2007). Flash Freezing and Cryosectioning E12.5 Mouse Brain. JoVE. 4. http://www.jove.com/index/Details.stp?ID=198, doi: 10.3791/198

Abstract

Protocol

1. Fix tissue in 4% paraformaldehyde in PBS for desired time.
2. Sucrose infuse tissue (cryoprotection)
    1. Make 30% sucrose solution in PBS w/v in 2059 tube.
    2. Rinse tissue 3x in PBS (~ 5 min with rocking).
    3. Place tissue in 30% sucrose solution. Tissue will not sink.
    4. Place the tissue in 4°C overnight, or until it has sunk.
3. Label appropriate size cryomold with information and orientation.
4. Fill cryomold with O.C.T. (avoid bubbles).
5. Transfer tissue to O.C.T. bath and coat it with O.C.T.
6. Transfer tissue to O.C.T. in cryomold.
7. Orient the tissue under microscope.
8. Pour liquid nitrogen into plastic Petri dish.
9. Quickly and carefully lower the tissue in cryomold into the nitrogen. (do not submerge the top of the cryomold.)
10. When the O.C.T. is solid white, place the frozen tissue into -80°C freezer for storage.
11. Equilibrate tissue to ~20°C for at least 30 min. prior to sectioning.

References

