FIXED BRAIN SECTIONING

PROTOCOL FOR CUTTING SLICES OF FIXED BRAIN TISSUE

Key to reading the protocol:

√ Rationale for procedural step

♠ Tips & Tricks

1. Deeply anesthetize animal, then transcardially perfuse with saline followed by 4% paraformaldehyde in 0.1 M phosphate buffer, pH 7.4.
   - √ Note that you can perfuse the animal in a variety of ways, including gravity-feed, pump, or push-syringe.
2. Remove brains by dissection and fix in 4% phosphate buffered paraformaldehyde at 4° C overnight.
   - √ Be careful not to fix your tissue for too long before cutting and processing it! Over-fixation will decrease good protein staining when you do immunohistochemistry. So don't forget to take out the fixed brain after 24 hours.
3. Pour out the paraformaldehyde and then cryoprotect the fixed brain with 30% sucrose buffer at 4° C overnight.
4. Pour out the 30% sucrose solution. If you are not sectioning the brain right away, store it in PBS until you are ready for cutting. If you are ready to section with the Compresstome®, rinse the brain in PBS first.
5. Select a section of the brain that you would like to take cut for slices.
6. Glue the tissue sample onto the Compresstome® specimen syringe.
7. Draw the syringe downward to bring the brain tissue core sample into the syringe.
8. Fill the syringe with 2% agarose (Sigma A-0701, low gelling point, incubated at ~37°C).
   - √ Order a Starter Kit or additional agarose or blades directly from our website at http://www.precisionary.com/starter-kit !

9. Cool the entire contents of the specimen syringe with the chilling block. The brain tissue is now embedded in agarose. The agarose will solidify enough for stable sectioning.
10. Load the specimen syringe onto the Compresstome® slicer.
11. The protocol is complete for preparing the fixed brain specimen for sectioning. Proceed from here with normal Compresstome® sectioning procedures.

♠ What are the optimal settings on the Compresstome® for cutting fixed brain slices? Try a speed (Advance) of 2 and an oscillation of 4-6. We have found that these parameters work best for obtaining superb brain slices with smooth surfaces without chattermarks.

References

*** Uses the Compresstome® for successful fixed brain slices.**

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