PRECISIONARY

SPINAL CORD SECTIONING

PROTOCOL FOR CUTTING SLICES OF SPINAL CORD

Key to reading the protocol:

√ Rationale for procedural step

♠ Tips & Tricks

This protocol can be adapted for either obtaining live slices of spinal cord (for electrophysiology and imaging) or fixed slices of spinal cord (for immunohistochemistry or in-situ hybridization).

1. For live spinal cord slices: First deeply anesthetize animal, then transcardially perfuse with saline to clear out blood in all organ systems. Dissect out the spinal cord and rapidly cool it in pre-chilled cutting solution.
2. For fixed spinal cord slices: First deeply anesthetize animal, then transcardially perfuse with saline to clear out blood in all organ systems, followed by 4% paraformaldehyde infused for 10 minutes. Dissect out the spinal cord and store it in 4% paraformaldehyde overnight, or for at least 24 hours before sectioning.
    - √ 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 spinal cord after 24 hours. Rinse the tissue in PBS and store it in 1X PBS until you are ready to cut slices with the Compresstome®.

Sectioning spinal cord slices with the Compresstome®:

3. Select a section of the spinal cord that you would like to take cut for slices.
4. Cut out a rectangular shape of solidified agarose or gelatin that is slightly larger than the spinal cord specimen itself.
5. Place (don't glue!) the spinal cord specimen on top of the rectangular piece of agarose/gelatin, and then glue one end on to the Compresstome® specimen syringe.
    - √ The rectangular piece of agarose/gelatin helps hold the spinal cord as it is embedded into the agarose and specimen syringe.

6. Draw the syringe downward to bring the spinal cord core sample into the syringe.
7. 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 !
8. Cool the entire contents of the specimen syringe with the chilling block. The spinal cord is now embedded in agarose. The agarose will solidify enough for stable sectioning.
9. Load the specimen syringe onto the Compresstome® slicer.
10. The protocol is complete for preparing the spinal cord specimen for sectioning. Proceed from here with normal Compresstome® sectioning procedures.

    ♠ What are the optimal settings on the Compresstome® for cutting spinal slices? For fixed tissue, try a speed (Advance) of 2 and an oscillation of 4-6. For live tissue, try a speed of 4 and an oscillation of 5-7. We have found that these parameters work best for obtaining superb spinal cord slices with smooth surfaces without chattermarks.

References

*** Uses the Compresstome® for successful spinal cord slices.**

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2. **Duncan J, Kersigo J, Gray B, Fritzsch B. Combining lipophilic dye, in situ hybridization, immunohistochemistry, and histology. J Vis Exp. 2011 Mar 17;(49).**

3. **Selever J, Kong JQ, Arenkiel BR. A rapid approach to high-resolution fluorescence imaging in semi-thick brain slices. J Vis Exp. 2011 Jul 26;(53).**

4. **Ting JT, Daigle TL, Chen Q, Feng G. Acute brain slice methods for adult and aging animals: application of targeted patch clamp analysis and optogenetics. Methods Mol Biol. 2014;1183:221-42.**