

## **Thin Sectioning of Slice Preparations for Immunohistochemistry**

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### Protocol

1. Prepare mold from tape for OCT platform.
2. Fill mold with OCT and freeze within cryostat or using crushed dry ice.
3. Remove tape from around frozen OCT platform.
4. Align marks on freezing chuck and cryostat mounting stage and lock in chuck.
5. Section through OCT platform until surface is flat.
6. Remove resurfaced OCT platform and place on cryostat freezing stage.
7. Place tissue sample (previously cryopreserved with 30% glycerol or sucrose in PBS) in OCT.
8. Prepare freezing column with outer ring projecting about 5 mm above top of column forming well for OCT.
9. Carefully position tissue sample onto center of freezing column surface and slowly add OCT until well is filled.
10. Surround freezing column with crushed dry ice. Tissue and OCT should completely freeze within 20-60 seconds.
11. As preparation increases in temperature, the outer ring can be removed while the sample remains frozen.
12. Slide sample off freezing column sideways and place in cryostat.
13. Place drop of OCT on surface of OCT platform and position specimen (tissue down) applying firm pressure. Specimen will quickly freeze onto OCT platform.
14. Secure chuck onto cryostat mounting stage with marks aligned.
15. Section through OCT superficial to the tissue specimen.
16. Thaw mount thin sections onto glass slides and store frozen or at room temperature.
17. Immunoreactions can be performed for tissue mounted on glass slides.
18. Reagent is pooled onto slide, can be gently agitated, and may be covered if light-sensitive.
19. Subsequent stages of the reaction are easily performed by inverting slide into waste receptacle, wicking the slide, and then applying the next reagent.