**Immunostaining Tissue:**

1. For postnatal and adult pancreas: Excise pancreas and fix in 4% PFA in PBS for 4 hours at 4°C.

2. Wash 3 times with PBS (15 min each)

3. Place in 30% Sucrose in PBS (Weight/volume) at 4°C overnight.

4. Next day, place tissue in OCT at Room temperature for at least 10 minutes.

5. Embed in OCT

6. Cryosection tissue 10-12 microns.

7. Let frozen section sit at Room temperature for 5 minutes.

8. Wash 3 time with PBS (5 minutes each, Room temp)

9. Permeabilize with 0.2% Triton X-100 in PBS for 15 minutes.

10. Wash 3 time with PBS (5 minutes each, Room temp)

11. Block at Room Temperature for 1 hour with 5% Normal Donley Serum in 1% BSA in PBS (w/v).

12. Add primary antibody to 1% BSA in PBS solution overnight.
   
   a. Nkx6.1 Rabbit Novus, NBP1-49672, 1:500 dilution
   b. Insulin guinea pig, DAKO, 1:1000 dilution

13. Next day, Wash 3 time with PBS (10 minutes each, Room temp)

14. Apply secondary antibody, 1:1000 dilution in 1% BSA in PBS. Room temperature for 2 hours. All Jackson immunoresearch
   
   a. Donkey anti rabbit Cy3
   b. Donkey anti guinea pig Cy2

15. Wash 3 time with PBS (10 minutes each, Room temp)

16. Apply DAPI Fluoromount to tissue and add coverslip