**Protocol staining Mouse Monoclonal CD8, Mouse Monoclonal Glucagon and Bovine anti-goat Somatostatin in Human Pancreas Frozen Tissue**

1. Human frozen pancreas tissue
2. Leave at room temperature for 5 min
3. Fix in acetone for 10 min
4. Wash tissue with PBS three times (5 min/time) at room temperature
5. Dry tissue at room temperature for 30-60 min
6. Circle tissue with liquid blocker super pap pen
7. Block tissue in 5% donkey serum at room temperature for 20 min
8. Add 1st antibodies: purified anti-human CD8a (clone RPA-T8, Mouse IgG1, k) at a ratio of antibody to PBS solution (1:200), somatostatin (D-20) sc-7819 goat polyclonal IgG (Santa Cruz Biotechnology) 1:200 in PBS
9. Incubate in a frig overnight
10. Wash tissue with PBS three times (5 min/time) at room temperature
11. Incubate biotin rat anti-mouse IgG1 monoclonal antibody for IHC (BD Pharmingen) in a concentration of 4 micro gram/ml in PBS and 2nd antibody bovine anti goat IgG Dy light 594 1:400 with tissue in PBS at room temperature for 30 min
12. Wash tissue with PBS three times (5 min/time) at room temperature
13. Incubate 1st antibodies with tissue: Monoclonal Anti-Glucagon antibody produced in mouse (Sigma) 1:200 and FITC Streptavidin (BD Pharmingen) 1:100 with tissue in PBS at room temperature for 30 min
14. Wash tissue with PBS three times (5 min/time) at room temperature
15. Incubate 2nd antibody donkey anti-mouse IgG Dy Light 649 1:400 at a concentration of 2.5% of donkey serum in PBS with tissue at room temperature for 30 min
16. Wash tissue with PBS three times (5 min/time) at room temperature
17. Add DAPI
18. Cover the stained tissue with cover slip
19. Take pictures