Immunofluorecent staining

1. Warm up the frozen slice in the room temperature for at least 10 min
2. Fix the sample with 4% PFA for 10 min
3. Wash with PBS 5min X 2
4. Block and permeablize the sample with Blocking buffers+ 0.1% Treatonx-100 for 30 min at RT
5. Add primary antibody (1:100 to 1:500) in the blocking buffer to surface of tissue. It is about 100ul solution per sample
6. Transfer the slides into the wet box and incubate at 4 degree for overnight. Or RT for 1-3 hour base on the result
7. Wash with PBST for 5min X 3
8. Add fluor conjugated secondary antibody (1:100-1:200) in the blocking buffer for 1hour at room temperature. To avoid solution evaporation, cover the slide with parafilm.
9. Wash with PBST for 5min X1
10. Conterstain with DAPI for 5min
11. Wash with PBST for 5min x3
12. (Optional) post fix with 4% PFA for 10min
13. Wash with PBST for 2min X3
14. Mount the slice with aqueous mounting medium (vector Shield) and put coverslides on the top
15. Seal the four sides of coverslide with colorless nail polish.

NOTE:
1. Sample can also be fixed with ice cold 100% methanol for 10 min or 100% aceton for 5-10 min base on different tissue and antibodies. For instance, the heat tissue fixed with acetone show better Desmin signal than with the other fixation.

Solution:
Blocking solution:
PBS + 5% normal serum (same species to secondary antibody) +1% BSA.
Blocking solution:
PBS+ 0.05% Tween20