Steps to follow for IHC on frozen sections:

1. Make PBST buffer--------------8 liters (Make sure MilliQ water is filled first, you will need that)
2. Make 1% BSA in PBST. Use a 50 ml tube and add 45 ml of PBST, weigh out 500 mg of BSA and add to the liquid and shake to dissolve. Label tube with date initial and contents
3. Make 0.03% H2O2: Take 250 microliters of 30% H2O2 and add to 250 milliliters of PBST
4. Place slides in 0.03% H2O2  for 30 minutes at room temperature and then wash in 3 changes of PBST
5. Make a humid chamber by pouring 250 ml of PBST into the base of the black plastic slide holder with cover
6. Place slides onto the holding tracks of the humid chamber and overlay each of the sections with 1% BSA/PBST and
7. Tip off the BSA/PBST and overlay with 0.1% avidin (white cap) for 15 minutes in the covered humid chamber, and then wash in 3 changes of PBST
8. overlay with 0.01% biotin (black cap) for 15 minutes in the covered humid chamber, and then wash in 3 changes of PBST
9. Immerse slides in 10% neutral buffered formalin in the hood for 30 minutes at room temperature and then wash in 3 changes of PBST
10. While the slides are fixing, get wet ice in ice bucket
11. Make the appropriate dilutions of primary reagent and set up in the ice bucket
12. overlay each of the sections with 1% BSA/PBST, tip off, and overlay with prepared primary reagents
13. Cover slides with parafilm
14. Incubate overnight at 4 degrees in the humid chamber, on the flat surface in the refrigerator.

The second day:

1. Remove humid chamber carefully from the refrigerator, take out each slide and place each in a tub of PBST
2. wash in 3 changes of PBST
3. Overlay with secondary reagent and incubate in the humid chamber for 30 minutes at room temperature and then wash in 3 changes of PBST
4. Overlay with tertiary reagent and incubate in the humid chamber for 30 minutes at room temperature and then wash in 3 changes of PBST
5. Make Substrate solution following manufacturer’s instructions and overlay onto each slide
6. Watch the negative control and if it starts turning color, wash all the slides and remove from the humid chamber and then wash in 3 changes of PBST
7. Immerse in nuclear counterstain and wash in 3 changes of PBST
8. Lay the slides out on the slide holder to dry before aqueous mounting for viewing and photography