# Immunohistochemical (IHC)-Ki67

1. Label slides with pencil or histo marker,
   *IHC: Ki67, IHC: - ctrl*
2. Deparaffinization and hydration to distilled water
3. Antigen Retrieval:
   - Fill 1.5 L of 1x citrate Buffer (pH6) solution into one PT Module tank.
   - Program PTM to 98°C for 20 minutes (will take about **1.170 hours** to reach temperature)
   - Press "Run" twice, make sure "Warm up" appears on the monitor, instead "preheat. Remove the slides from the slide holder and place in the container with 1xPBS
4. Remove the excess buffer around the tissue with a Kimwipe. Then draw a circle around the tissue with a hydrophobic pen.
5. Place slide on the staining tray and add enough drops of washing buffer to cover the tissue to prevent it from drying out.
6. Remove washing buffer by tapping off the excess solution.
7. Apply enough of "Dual Endogenous Enzyme Block (DEEB)" to cover the tissue, incubate for **10 min** @ room temperature
8. Tap off the "Dual Endogenous Enzyme Block (DEEB)" onto paper towels. Apply washing buffer for **5 minutes** *(This process is known as rinsing)*
9. Tap off washing buffer
10. Preparing primary antibody:
    - Centrifuge anti-Ki67 tube
    - Dilute anti-Ki67 (1:200) with "Antibody dilutant"
    - Apply primary antibody ki67 to testing slide and the positive control slide.
    - Apply 1 x PBS on the negative control slide. Do not apply the primary antibody (Ki67) on the negative control slide.
    - Carefully transfer the slides into the 37°C humid chamber and incubate for **1 hour or 4°C overnight.**
11. Tap off the solution on the slides. Rinse with "washing buffer" for **5 minutes.**
12. Repeat step 11, two times
13. Tap off washing buffer. Apply enough drops of "Labeled Polymer Anti-rabbit" to cover the tissue, incubate for **30 minutes** at room temp.
14. Tap off excess polymer and apply washing buffer for **5 minutes or longer**
15. In an Eppendorf tube, add 1 mL of "DAB+ Substrate Buffer" and 1 drop of **"**DAB+ chromogen". Vortex the solution so it can mix well.
16. Tap excess washing buffer off the slide. Apply DAB + substrate chromogen and incubate for **10 minutes** at room temperature
17. Rinse DAB+ with distilled water.
18. Dip the slides 1 or 2 times into hematoxylin and wash with tap water. Then dips 3 times into the bluing reagent and wash with tap water.
19. Undergo dehydration and clearing steps.
20. Coverslip with mounting media