**Immunofluorescence for paraffin-embedded tissue sections**

**DAY 1**

1) Remove paraffin and rehydrate the tissue sections

   a) Xylene ------ 2x 5min RT (**Perform this step in the hood**)  

   Note: Air dry xylene slides in the hood and circle the tissue sections with Hydrophobic Barrier PAP Pen  

   b) 100% Ethanol------ 2-3 min RT  
   c) 100% Ethanol------ 2-3 min RT  
   d) 100% Ethanol------ 2-3 min RT  
   e) 95% Ethanol------ 2-3 min RT  
   f) 70% Ethanol ------ 2-3 min RT  
   g) 50% Ethanol------ 2-3 min RT  
   h) 1XPBS------ 5 min RT  

2) Antigen retrieval (Heat-based antigen retrieval)

   a) Preheat the steamer  
   b) Preheat the antigen unmasking working solution in a microwave for 10 mins or by heating the solution over a Bunsen burner until boiling  

   Note: prepare 450ml antigen unmasking working solution (4.2ml antigen unmasking stock solution + 450 ml dH₂O)  

   c) Transfer the boiled antigen unmasking solution to the steamer and steam the slides in the antigen unmasking solution for ~15-20mins
d) After steaming is complete, transfer the slides to FRESH 1XPBS solution

3) Block unspecific staining
   a) Add blocking buffer to the sides (~ 200µl per slide). Make sure the blocking buffer have covered all the specimen
   b) Incubate the tissue sections with blocking buffer (stored in the freezer) inside a humid chamber for 1h at RT
   c) Proceed directly to step 4. **DO NOT WASH**

4) Primary antibody
   a) Dilute the primary antibody in blocking buffer at concentrations depend on the antibodies used
   b) Add primary antibody to the sides (~ 150µl)
   c) Incubate the tissue sections with primary antibody O/N at 4°C inside a humid chamber
   d) Wash in PBST 5’X3
      Note: Prepare 500ml PBST (50 ml 10xPBS + 0.5 ml Tween 20 + 449.5 ml dH20)

DAY 2

5) Secondary antibody ****LIGHT SENSITIVE****
   a) Dilute the secondary antibody in blocking buffer (1:200)
   b) Add secondary antibody to the sides (~ 150µl per slide)
   c) Incubate the tissue sections with the secondary antibody inside a humid chamber for 2h at RT
d) Wash the slides in PBST 5’ (COVER WITH ALUMINUM FOIL)

6) DAPI staining **LIGHT SENSITIVE**

a) Add DAPI to the slides (~ 200µl per slide)

b) Incubate the tissue sections with DAPI (stored in the fridge) for 5 mins inside the humid chamber

c) Wash the slides in PBST for 5min (COVER WITH ALUMINUM FOIL)

d) Mount the slides for imaging