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 dH_20)

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 4^0C 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