Immunofluorescence for mouse incisor cryosections

**DAY 1**

1) 1XPBST------ 5 min RT 3 times

Note: Prepare 500ml PBST (50 ml 10xPBS + 0.5 ml Tween 20 + 449.5 ml dH20)

2) Block unspecific staining
   a) Add blocking buffer to the sides (~ 200μl per slide). Make sure the blocking buffer covers the entire 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 3. **DO NOT WASH**

3) Primary antibody
   a) Dilute the primary antibody in blocking buffer (concentration depends on the antibody 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

**DAY 2**

4) Secondary antibody ****LIGHT SENSITIVE****
   a) Wash in PBST 5’X3
   b) Dilute the secondary antibody in blocking buffer (1:200)
c) Add secondary antibody to the sides (~ 150μl per slide)

d) Incubate the tissue sections with the secondary antibody inside a humid chamber for 2h at RT.

e) Wash the slides in PBST 5’ (COVER WITH ALUMINUM FOIL)

5) 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