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 dH_20)

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