**Immunostaining protocol for zebrafish sections (two-day protocol)**

Make sure that the slides are dried overnight

**Day1**

1. Put tissue in Coplin jar and cover it in PBS for 5 minutes at RT
2. Antigen retrieval
* Get rice cooker out and fill it with 3 to 4 cm water. Do not turn it on yet
* Put slides in Coplin jar and cover with 10 mM sodium citrate (pH 6)
* Put the open Coplin jar in the rice cooker and close the lid of the rice cooker
* Turn on the rice cooker to the highest setting for 20 min
1. Take the Coplin jar out of rice cooker and let it cool down to RT for 30 minutes
2. Wash the slides three times at RT with PBST (0.1% triton X) for 5 minutes each
3. Blocking solution (1% BSA, 10% Goat Serum in PBST)
* Put wet cloth in the slide tray
* Take out two slides at the time and dry them
* Put per slide 150 µl blocking solution next to the tissue
* Cover it with parafilm
* Leave on bench for 1 hour covered up
1. Primary antibodies solution
* Whilst waiting for the blocking solution make the primary antibody staining
* Per slide use 150 µl blocking solution and mix it with desired antibody (be careful with the ratio)
1. Apply primary antibody
* Three slides at the time
* Remove the parafilm and put to the side with sticky side up as it will be used again
* Remove excess water
* Put 150 µl from the primary antibody solution on each slide
* Cover the slides with parafilm again
* Put in fridge (4 degrees Celsius) for overnight

**Day 2**

1. Take samples out of fridge and wash them at room temperature (RT) in Coplin jar three times with PBS for 20 minutes each
2. Make secondary antibody solution
* Use 150 µl blocking solution per slide and add the desired secondary antibody. The ratio is 1:1000 (total volume 150 µl; rabbit 488, mouse 647)
1. Apply secondary antibody
* Take two slides at a time, dry them, and put them in the tray (it still has the wet cloth in it)
* Apply 150 µl of the secondary antibody solution
* Cover with parafilm
* Caution: slides should not touch!
1. Close the slide box and leave at RT for two hours
2. Wash slides at RT with PBS for 20 minutes twice (cover the Coplin jar)
3. Wash slides at RT with PBS with added DAPI (40 µl) for 20 minutes
4. Mounting
* Take out wet cloth of slide tray
* Take out two slides at the time, dry them and put them in the slide tray
* Add three drops of ‘mounting medium with DAPI fluoroshield’ next to sample
* Pop the bubbles if needed
* Then roll on the Deckgläser (24mm x 60mm)
* Caution: do not move Deckgläser
1. Carefully put them in the draw (or any dark space at RT) with lid open