Day 1:

Preparing the slides/plates/dishes:

· Pipette about 200µl Poly-L-Lysine to cover the area on the dish. Leave it for 15 minutes.

· Remove and give a water wash.

· Put them on 55-60°C oven for about 15 minutes to dry it.

Formaldehyde fixation

· Dissect about an average 5 pairs of tubules per plate.

· Just before transferring the tubules on the dish, add about 100µl 1xPBS on the plate.

· Transfer the tubules as quickly as possible and try to stick them on the dish with glass rod or forceps without breaking the tubules.

· Remove PBS with a pipette and add (fix) with 4% Paraformaldehyde in PBS. (20 minutes)

· On the shaker, give 3x10 min. washes with 1xPBS.

· Permeabilise in **PBT** (PBS+0.3%triton) for 30 minutes.

· Block with **PAT** [=PBT+10% goat serum] at RT for 3 hours.

· Incubate o/n at 4°C with 1° Antibody diluted in PAT.

Or

Heat fixation

· Put E-Wash (10mM NaCl, 0.1% Triton) at 80°C on heating block for about 20 minutes. Make 100µl aliquots (1aliquot=1 dish) in small eppendorfs to put on heating block.

· Fix for 8 seconds in E-wash at 80°C. Remove PBS from dishes and add 100µl E-wash from 80°C and put them on heating block for exactly 8 sec. Do one dish at a time.

· Have some chilled E-wash ready in a falcon tube and pour this on the dish after 8 sec. on the heating block. Incubate the tubules on ice for about 2 minutes.

· Remove all E-Wash and pour some methanol to cover the bottom surface of the dish. 2 hrs.

· Remove methanol and rinse once with PBT. Wash with PBT 2x30 mins. on shaker.

· Incubate o/n at 4°C with 1° Antibody diluted in PBT+4% goat serum.

Day 2:

· Give 3x15minute washes with PBT (4x1hr with PBT for heat fixation protocol).

· Blocked with PAT, 2-4 hours (PBT+4% goat serum for heat fixation).

· Incubate o/n with 2° Antibody diluted in PAT (PBT + 4% goat serum for heat fixation) at 4°C. Cover it in an aluminium foil.

Day 3:

· Give washes with PBT every 15 minutes for 2 hours (3x1hr washes for heat fixation protocol).

· Incubate with DAPi for 1 min.

· Wash with PBS (3x15 mins.).

· Mount in vectashield.

· Parafilm the plates and put in fridge.