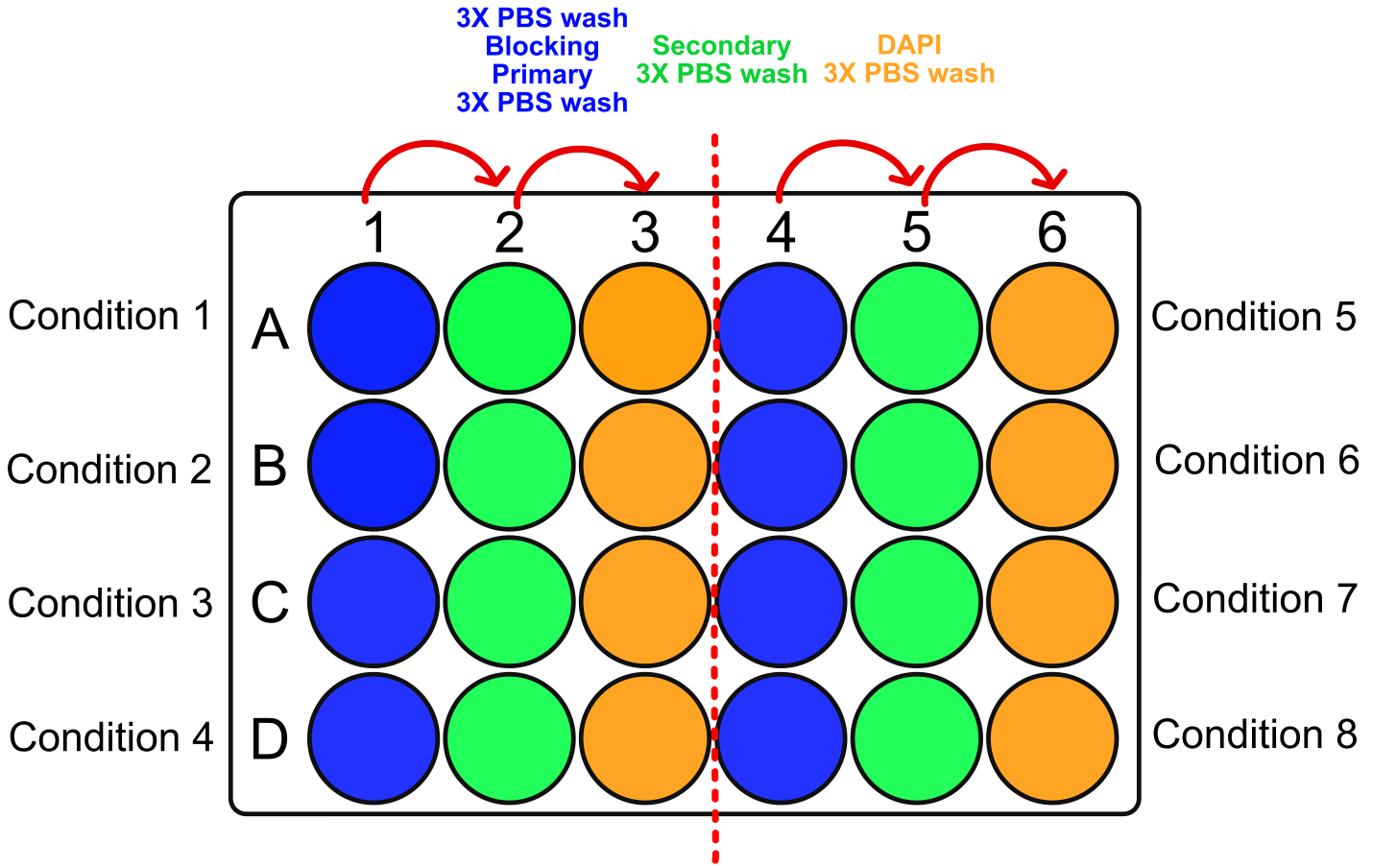
Free-floating immunofluorescence



Scheme of the step for free-floating immunofluorescence. The following protocol refers to the wells in this scheme.

Day 1 : Blocking and primary antibody

Blocking solution : You need a 10% normal donkey serum (NDS) from the freezer and PBT (1X PBS + 0.05% Tween) that is kept at room temperature (RT). Transfert 1,35 mL of PBT + 150 uL of NDS in an ependorf.

1. Open a new 24-well plate and fill the wells you will use with solution.
2. If the brain slices were in PBS for a few days, it is worth to wash them before starting. If this is not the case, go to the next step. If this is the case,
    1. add 300 uL per (blue) well of fresh 1X PBS in the wells you will use.
    2. transfert the brain slices you want to stain with a thin paint brush (in blue wells). Ideally, we want 4-5 brain slices per well.
    3. Wash 3 times with **1X PBS for 5 minutes each at RT** in the dark on the rotation machine-thingy at 180-200 rpm.

3. Put 300 uL of blocking solution in the (blue) wells.
4. If no PBS wash was done previous to the blocking, transfert the brain slices you want to stain with a thin paint brush (in blue wells). Ideally, we want 4-5 brain slices per well. Otherwise, go to the next step.
5. Incubate in 300 uL of blocking solution for **30 minutes at RT** in the dark on the rotation machine-thingy at 180-200 rpm.
6. Change the 300 uL of blocking solution for 250 uL of blocking solution.
7. Add the primary antibodies to the blocking solution in the (blue) wells.
8. Incubate **overnight (O/N, 16-20 hours) at RT** in the dark on the rotation machine-thingy at 180-200 rpm.

Day 2 : Secondary antibody and mounting

1. Wash 3 times with **1X PBS for 5 minutes each at RT** in the dark on the rotation machine-thingy at 180-200 rpm.
2. Add 250 uL of blocking solution in the next (green) wells.
3. Transfert the brain slices to the next well (green) with a thin paint brush.
4. Add the secondary antibodies to the blocking solution in the (green) wells.
5. Incubate for **2 hours at RT** in the dark on the rotation machine-thingy at 180-200 rpm.
6. Wash 3 times with **1X PBS for 5 minutes each at RT** in the dark on the rotation machine-thingy at 180-200 rpm.
7. Add 250 uL of DAPI from the fridge in the next (orange) wells.
8. Transfert the brain slices to the next well (orange) with a thin paint brush.
9. Incubate in DAPI for **3 minutes at RT** in the dark on the rotation machine-thingy at 180-200 rpm.
10. Wash 3 times with **1X PBS for 5 minutes each at RT** in the dark on the rotation machine-thingy at 180-200 rpm.
11. Transpose the brain slices from the (orange) wells to a microscope slide with a thin paint brush.
12. Let the slices dry for **20-30 minutes**.
    When the brain slice is flat on the microscope slide, try to tilt the slide to be able to drain the liquid with a KimWipe without touching the tissue. This will help to reduce the drying time.
13. Put a drop of mounting medium (Prolong) on each brain slice.
14. Mount the samples with a coverslip.
    Be careful: we don't want any bubbles! You can use a pipette tip to push the bubbles away.
15. Leave the slides in the dark for **24 hours at RT**.

Day 3 : Storage

Store the mounted samples in the dark at -20°C or 4°C until imaging.