**Immunofluorescence staining**

**1. Fixation of the cells:**

**Strategy 1**: i.e., HEK cells

1. Remove medium
2. Wash with 1X PBS
3. Remove PBS and add 4% PFA to the cells [for a coverslip in a 24 multiwell use at least 300µl/well]
4. Incubate 10 minutes at RT
5. Collect PFA
6. Wash 3x with 1X PBS for 5min at RT

**Strategy 2:** i.e., neurons

1. Add the same volume of 8% PFA as medium is in the well to the well
2. Incubate 10min at RT
3. Collect PFA in a falcon
4. Wash in PBS 1X (2x, 5min, RT)

-> Storage until ICC: keep coverslips in PBS 1X, seal the plate with parafilm and store at 4°C

**2. Blocking and permeabilization:**

1. Remove 1X PBS
2. Add 300µl/well of blocking solution   
   (10% NGS [normal goat serum] in PBS + Triton X-100 0,1%, filter the solution before using it)
3. Incubate at least 1h at RT

**3. Staining:**

**Day1:**

1. prepare antibody in blocking solution containing 5% NGS
2. Put a drop (50µl) of Primary antibody solution on the parafilm surface
3. Remove the coverslips from the plate and gently put it upside-down on the antibody drop.
4. Incubate O/N at 4°C

**Day2:**

1. Wash (3x, 5min) in PBS + Triton X-100 0,1%
2. Prepare secondary antibody in blocking solution containing 5% NGS (keep in the dark)
3. Put a drop (50µl) of secondary antibody solution on the parafilm
4. Take the coverslips and put it upside-down on the antibody drop
5. Incubate 1h at RT in the dark
6. Transfer the coverslip to a 24-well containing 500 µl 1X PBS + Triton X-100 0,1%
7. Incubate for 5 min at RT (in the dark)
8. Dilute DAPI 1:10000 in 1X PBS
9. Remove PBS and incubate with DAPI for 5 min at RT in the dark
10. Wash twice with 1X PBS
11. Mount the slides:

* Put a drop (10µl) of DAKO mounting reagent on the slide
* Take out the coverslip from the plate
* Dry it by gently tapping the coverslip’s edge on a lens-cleaner tissue
* Gently put the coverslips upside-down on the DAKO drop
* Leave it dry (24h, **DARK**, RT)

1. store in a slide-box at 4°C

**Recipes and products:**

|  |
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| **8% Paraformaldehyde (PFA)** |
| 20g PFA  0,5ml 1M NaOH  100ml 1xPBS |
| -> heat to ~60°C to dilute. Then filter through folded filters into new cylinder. |
| Adjust pH 7,4 (normally its ~7,38 without adding something) |
| Fill up to 250ml with 1xPBS |

**Dako Mounting Medium** (Dako, S302380-2):

**NGS (normal-goat serum)** (Biozol, VEC-S-1000)  
 prepare aliquots out of stock and store in -20°C

-> filter before use to avoid contamination

**DAPI (DAPI (4',6-Diamidino-2-Phenylindole, Dilactate)** (Biolegend, 422801):

Dissolve the content in 2 ml deionized water (DAPI concentration 10.9 mM)