Immunofluorenscence (IF) staining of human iPSCs and iPSC-CMs
(Stillitano's Lab)

Immunostaining of iPSCs and iPSC-CMs.

1. Wash the cells in **cold** PBS with Ca2+ and Mg2+.
2. Fix with 4% PFA for 10-15 min at RT.
3. Wash with PBS. (Cells can be stored at 4°C in PBS before staining).
4. Remove PBS, add blocking buffer. Incubate for ~1h at RT.
5. Remove blocking buffer and add primary antibody diluted in blocking buffer (iPSCs pluripotent markers: Nanog, Oct-4, SSEA1, and TRA-1-60, dilution 1:100. Cardiac specific markers: cTnT, Serca2a, PLN, dilution 1:200).
6. Incubate overnight at 4°C.
7. Wash 3 times with PBS, 5 minutes each.
8. Add secondary antibody 1:1000 (Alexa Fluor 488 (green) or Alexa Fluor 555 (red)) in blocking buffer.
9. Cover with aluminum foil to protect it from light and incubate for 1h at RT (keep the aluminum foil for all the following steps).
10. Wash 3 times with PBS, 5 minutes each.
11. Add a sufficient volume of DAPI Working Solution (1µg/ml) to completely cover the sample and incubate for 10-15 min.
12. Wash sample thoroughly with PBS to remove excess DAPI.
13. Store in PBS at 4°C for 4-8 weeks or proceed to mounting if the cells are plated on glass coverslips.

MOUNTING

Put a drop of mounting medium (VECTASHIELD without DAPI) in the center of the coverslip. Put your slide upside-down and bring it down on the coverslip until it touches the drop of mounting medium and attaches to the coverslip. The medium will spread gradually to the edges of the coverslip without bubbles. Then you can turn the slide upside-up and press it gently with a paper wipe to absorb any extra medium from the edges of the coverslip.

Solutions

Blocking Buffer (2%BSA/2%FBS/0.05% NP-40 in PBS)

DAPI (Thermo Scientific, 10 mg)

1. **DAPI Stock Solution**
   Dissolve DAPI in ultrapure water to 1 mg/ml. Stock solution is stable for several months and repeated use if stored protected from light at -20°C.
2. **DAPI Working Solution**
   Dilute the DAPI Stock Solution 1:1,000 in ultrapure water or PBS (1 µg/ml DAPI).