**Immunofluorescent (IF)**

1. Treat cell 1M per well (24hrs).
2. Withdraw all the cell into 15mL falcon and add HBSS 10ml.
3. Centrifuge 1500- 5 mins and withdraw the supernatant.
4. Resuspend with 1ml HBSS and transfer into Eppendorf.
5. Count cell and normalized all samples to 0.5M.(500,000cell /mL) by adding PBS.
6. Put in icebox.
7. Withdraw 100uL ~~150uL~~ and out into cytospin well.
8. After cytospin, take out the slides and look under the microscope.
9. Draw the circle line and pipette ~~~75uL PFA 4% into the sample.~~

Fix with PFA 4% in the container 100ml for 15 mins. Can reuse the PFA solution in the container.

1. ~~Do it in the 4C room and keep in the cupboard for 1 hour.~~
2. Dry by tapping on the wipe. Pour in 1x PBS to wash slides. Repeat wash for 3-4x.
3. Permeabilize using PBS/0.5% Triton-X for 10 mins.
4. Blocking using PBS/0.5% Triton-X+ 5% Lamb Serum for 1 hour at RT in the black box. Use 5% BSA ini PBST
5. -
6. Put in primary antibody according to the ratio (dilute with blocking agent) overnight.
7. Wash the next day and put in secondary antibody.
8. Wash
9. Check under microscope after mounted the slides.
10. Let it airdry overnight at RT in dark.
11. Next day put in 4C if confocal was arrange later.