EXPERIMENT NO: 2
DATE : **27 - 28 March 2018 and 4 April 2017**

CHARACTERISATION of CELLS
(staining for mitochondria, cytoskeleton and nucleus)

Material you need:

- cells (sheep skin fibroblasts)
- around 20 ml of PBS (in 50 ml tube)
- 1ml of 4% Paraformaldehyde (transfer into eppendorf)
- 1ml of 0.1% Triton X- 100 (transfer into eppendorf)

- 1ml of Mitotracker Green solution (transfer into eppendorf and cover with aluminium foil) - for mitochondria
- 1ml of Phalloidin solution (transfer into eppendorf, cover with aluminium foil) - for cytoskeleton
- 1ml of Hoechst solution (transfer into eppendorf, cover with aluminium foil) - for nucleus

- Pasteur pipet
- Piece of aluminium foil
- trash container

- Needle
- Pincette
- 1 big glass slide

Procedure

1. Prepare work place - take all needed materials.
2. Observed cells under inverted microscope.
3. Discard culture medium using Pasteur pipet.
4. Add 1ml of PBS.
5. Wash cells with PBS then aspirate and trash
6. Add 1ml of medium contained Mitotracker Green (50 nM working solution) (**FOR MITOCHONDRIA STAINING**)
7. Incubate 15 min in 37°C covered from light (light sensitive).
8. Discard medium contain Mitotracker Green.
9. Add 1ml of PBS and wash cells for 2-3 min. Repeat 2 time.
10. Add 1ml of 4% paraformaldehyde (PFA). **Careful - PFA is toxic**.
11. Incubate 10 min at 37°C (incubator).
12. Carefully aspirate paraformaldehyde and trash.
13. Add 1ml of PBS - wash for 3 min.
14. Repeat washing 3 times.
15. Add 1ml of 0.1% Triton - X - 100 incubate at room temperature (RT) for 10 min (cover from light).
16. Aspirate 0.1% Triton - X- 100 and trash.
17. Add 1ml of PBS - wash for 3 min.
18. Repeat washing 3 times.
19. Add 1ml of medium contains 500ng/ml of Phalloidin conjugated with FITC (green) (**F-actin staining**).
20. Incubate at room temperature for 30 min (covered from light).

21. Discard medium with Phalloidin.
22. Add 1ml of PBS - wash for 3 min.
23. Repeat washing 3 times.
24. Add 1ml of PBS contains 5ug/ml of Hoechst (**NUCLEAR STAINING**)
25. Incubate for 5min cover from light.
26. Aspirate PBS containing Hoechst.
27. Add 1ml of fresh PBS
28. Wash quickly 2 times.
29. Take big glass slide.
30. Wash glass with 70% ethanol
31. Add small drop of mounting medium in the centre of glass slide.
32. Take out cover glass with cells (use needle and/or pincette) and put on the drop of the mounting medium
33. !!!!!!!!!! **BE CAREFUL** remember that cells grow on the top of the glass. Put cover glass with cells that cells touch mounting medium - **ASK for ASSISTANCE**!!
34. Observe under fluorescent microscope.
35. Mitochondria - green “spots”, Actin - green “line”, nucleus - blue)
36. For long storage cover with nail polish. Cover from light.