PROTOCOL 3. FREEZING CELLS

- Cells

Reagents:
- Trypsin (eppendorf)
- PBS (50 ml Tube)
- MEM + 10% FBS (15ml tube)
- Freeze medium (20% FBS, 20% DMSO in MEM medium) (eppendorf)

Materials:
- 1x cry-tube
- Few Pasteur pipets
- 15 ml tube
- Trash container

PROCEDURE:
1. Prepare your workplace
2. Aspirate culture medium from the cells and trash
3. Add 2 ml of PBS
4. Wash carefully (rotate left and right couple of times)
5. Aspirate PBS and trash
6. Add 1ml of Trypsin - distribute well in the culture dish, make sure that cover all cells
7. Incubate 3 min at 37°C
8. Observe under the microscope if all cells detached from the surface.
9. Add 5ml of MEM + 10% FBS to block activity of the tripsin
10. Transfer all (medium/trypsin and cells) to the 15 ml tube
11. Spin for 5min at 1200 rpm.
12. Aspirate supernatant and resuspend cells in 0.5 ml of MEM + 10% FBS
13. Transfer cells (0.5 ml) into the cryo-vials.
14. Add DROP BY DROP 0.5 ml of freezing medium.
15. Close tap.
16. Write your name, date and cell type on the cryo vial.
17. Mix carefully (up and down) and transfer into MISTER-FROSTY.
18. Place at -80°C overnight.