FREEZING AND THAWING METHOD

Freezing

1. Prepare the Freezing solution (filtered FBS + 10% DMSO) and kept it on ice;
2. Wash the cells with PBS and centrifuge at 1200 rpm;
3. Dilute the pellet, consisting of a maximum of $1x10^6$cells/ml, in 500 µl of culture medium;
4. Dispense the pellet so diluted in a cryovial (specify in the label the cell code, the freezing date and, depending on the cell type, also the passage);
5. Add 500 µl of cold freezing solution to the cryovial and ensure that the solution is homogeneous;
6. Transfer immediately to the green container at 4° C and store at - 80°C for at least 4 hours;
7. Then transfer the cryovial from the green container to the purple one (storage container), that is already at -80° C (bring the green container back to 4° C in order to use it for subsequent freezing);
8. Leave at -80° C for 24 hours;
9. Transfer the cryovial at -196° C.

Thawing

1. Switch on the thawing tool (ThawSTAR Automated Cell Twawing System-BioCision);
2. Place the cryovial from the liquid nitrogen container to the green conveyor at -80° C;
3. Place 1 ml of filtered FBS in a 15 ml sterile tube;
4. Insert the cryovial into the instrument, after about 1 minute an acoustic signal will be emitted and the cryovial will be automatically ejected;
5. Slowly dispense the suspension into the 15 ml tube containing the serum;
6. Add at least 10 ml of medium;
7. Centrifuge at 1000 rpm for 5 min;
8. Wash in culture medium;
9. Centrifuge at 1000 rpm for 5 min and finally plate cells with cell culture specific medium.

Autor: L. Mangiapane **15/09/2015** **Approved: Giorgio Stassi**