

<p>Biomechanical Orthopedics Laboratory EPFL-HOSR & Orthopedic Cell Therapy Unit CHUV</p> <p>SOP 08-07</p> <p>Page 1 of 2</p>	<p>Cell freezing</p> <p>Version 4</p>	<p>Creation date : 11.11.03 Created by : AK Modification date :18.12.07 Modified by : LALA</p>
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Materials :

- Dimethyl Sulfoxide (DMSO) (Sigma, #D5879)
- Phosphate Buffered Saline (PBS without Ca⁺⁺ / Mg⁺⁺) 500 ml (Invitrogen #100110-15) at RT
- Trypsin-EDTA (1X) 500 ml -> Aliquot x ml (Invitrogen #25300-62) at -20°C
- DMEM (with L-Glutamine, 4,5 g/l D-Glucose and Sodium pyruvate) 500 ml (Invitrogen, #41966-29) at 4°C
- Fetal Bovine Serum (FBS) 500 ml -> aliquot 50 ml (Sigma, #F7524) at -20°C
- L-Glutamine (200 mM ;100x) 100ml -> aliquot 5 ml (Invitrogen #25030-024) at -20°C

Culture medium preparation : (for 500 ml)

- Add 50 ml FBS (10%), 5ml L-Glutamine (1%) to the 500 ml bottle of DMEM
- Filter, if necessary, the medium with Stericup system (Milian SCGPU05RE)
- Heat the culture medium, PBS and Trypsin at RT or in 37°C water bath

Freezing medium preparation : (for 10ml)

- 5 ml culture medium (50%)
- Add 4 ml FBS (40%)
- Add 1 ml DMSO (10%)

Freezing Procedure : (flask 25 cm²) / (flask 80 cm²)

1. Clean and prepare inside the hood all instruments
2. Check the confluence of cells with the microscope and the sterility
3. Throw away the culture medium
4. Wash 1x 3 ml / (**5 ml**) PBS the cells and throw away the PBS
5. Put 1 ml / (**2 ml**) Trypsin-EDTA (1x)
6. Wait until the cells detach in the incubator at 37°C, tap gently and check under the microscope
7. Collect the cells in the tube
8. Wash the flask with 2 ml / (**4 ml**) culture medium and collect the remaining cells in the tube
9. Centrifuge 5 min at 200 g at RT for tube of 15 ml or 10 min for tube of 50 ml
10. Aspirate the supernatant

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11. Homogenize gently the cells with 1 ml Freezing medium and put in a cryogenic tube (Milian #QSP-520-5)
12. Put the cryogenic tube in the Nalgene box (RéactoLab #1060295) at -80°C overnight
13. Place the cryogenic tube in the liquid nitrogen tank at the location defined in the cell logbook
14. Update cell logbook