Materials :

- Phosphate Buffered Saline (PBS without Ca++ / Mg++) 500 ml (Invitrogen #100110-15) at RT
- Trypsin-EDTA (1X) 500 ml -> Aliquot 10 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 DMEM bottle
- 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

Procedure : (flask 25 cm^2) / **(flask 80 cm^2)**

1. Clean and prepare inside the hood and all instruments
2. Check the conflence 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 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
11. Add fresh culture medium x ml for x news flasks and gently homogenize cell mixture for 30 sec
12. Put 4 ml / **(10 ml)** of cell suspension in the news flasks
13. Place in the incubator at 37°C and 5% CO_2.
14. Culture medium changes 2-3 times per week

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