Cell Culture/Cell Passage

Introduction

This protocol will help you to know which material you need and how do you process to successfully culture your cell

Material

- 500ml of DMEM (Invitrogen)
- 50 ml of FBS
- HeLa cells or MCF-7 cells
- T175 flasks
- Gibco Trypsin-EDTA (0,5%)
- PBS

Culture Cell :

1- Prepare media by Mixing the 500ml of DMSO and 50mL of FBS and filtering it.

2- Count your cells to plan how many cells you will initially put inside the flask.

3- add the desired volume in 2ml of media. The number of cells needed may differ with the cell line you are working with.

4- Put 28 mL of media inside the T175 flask

5- Put your cell + media inside the T175 Flask. And put the flask in the incubator at 37°c and 5% of CO2

Cell Passage

Prerequisite : Cell at 80% of confluency

1- Aspirate the medium (put your Pasteur tip on one of the top corners of the flask so you don't touch the cells)

2- Add 5ml of PBS to wash the cells and remove the dead ones (move laterally the flask to wash all the cells)
3- Aspirate the PBS
4- Add 5ml of PBs
5- Aspirate
6- Add 3mL of PBS and 1mL of trypsin
7- Incubate at 37°C for 5-10min
8- Go back under the hood: tap the flask to be sure you've detached all the cells
9- Add 4mL of medium, with your 10mL pipette, wash the flask and homogenise the solution (DMEM stops the trypsin action). Avoid bubbles as much as you can
10- Transfer the 8mL in a 15mL Falcon tube
11- Spin for 4min at 1200rpm, 20°C
12- Aspirate the supernatant
13- Tap the pellet to break it and disperse the cells
14- Add 1mL medium with 5mL pipette
15- Resuspend by adding 9ml of medium
16- Count the cells:
17- Label your T175 flask
18- Add 29.5 ml of medium + 550 µL of cells