SA

11.02.15

Washing Cell from medium: taking cells from Pa to P(a+1)

1. Take cells from incubator and remove medium.
2. Add 5mL-PBS to wash (add & remove)
3. Add 4mL-Tripsin and leave it 5 min in incubator
4. Take 4mL-Tripsin in a tube and Add 4mL-TNS and 4mL-PBS
5. Centrifuge at 1000rpm during 5min or always at 450RCF during 8min (ACV).
6. Remove liquid (Attention WITH CELLS!)
7. Add 4mL medium (EMB-2 complete)
8. Cell counting:
   1. Clean coverslip with ethanol and count 1-big square (Average x4)

Example1: 31 cells counted from a 4mL medium solution.

Total amount of cells are 31x10e4 **\* 4**(from 4mL sol.) = 1’240.000c

Rules: Average for confluence cultures 3M cells

Percentage of minimum confluence 75%

Flask surface 75cm3 (Normal flask). Volume in flask 15mL. Cell confluence 3M

Flask surface 56cm3 (6well plate). Volume in flask 11.2mL. Cell confluence 2.3M

Cell count when P1 to P2 is 600.000cells in 15mL

Each/24 well surface 2cm2. Volume per well 500uL

So, the ideal 75% in a 75cm2 flask should be 2.1M (=3M\*0.75). Means 28000 cells per cm2 (=2.1M/75).

If we have 2cm2 per well, and we intend to run 6 wells, then our area is 12cm2. This means 336000 cells/cm2.

Then 336.000 cells X

1’24.000 cells 4mL

X = 1.08mL

Because we want to fill 6 wells of 500uL each, our total desired volume is 3mL. So, we need to add 1.92mL EMB-2 to the previous calculation to complete 3mL.

***Otro caso:*** contadas 850000c. Minimum confluency in 24well plate is 28000c. Then 850000/28000 = 20.7 which aprox. 20well maximum with this count. As 500uL is the max. amount used to seed cells, then 20\*500=10000uL or 10mL. Finally, centrifugate 4mL tube, remove liquid and resuspend in 10mL of medium.

1. New tube: add 4mL medium + Calculated cell solution
   1. For flask 15mL of medium
   2. For 24well plate: 500uL

**Preparation of 24 well flask**

1. Insert cristal-wafer coverslip
2. Coating with 200uL in Gelatin 2%
3. Leave it in the incubator for at least 30minutes before start seeding

*To seed cells*

1. Remove Gelatin and wash with PBS (2x)
2. Add 500uL of medium with cells

**Fix sample for Imaging (In case that the imaging is required for other day it can be freeze at 5 C after finishing the procedure)**

1. Collect required items: PBS, PFL (500mL Falcon tube from common fridge – **warm it up)** and samples in well plates.
2. Remove medium (use bench work suction pump)
3. Add 500uL/well of PBS (no shaken is required. When adding, release carefully PBS in wall)
4. Add 500uL/well of RT PFL 4% and **immediately Step 5**
5. Shake samples during exact 15minutes in the shaker plate (ACV bench)
6. Wash samples 3x5’ with PBS (careful with PBS release)
7. Leave 500uL PBS and cover the samples with Parafilm to keep edges protected from contamination.
8. Ready to Image or Keep it in fridge and image later.

Mounting cells

Use cristal slides and add Vecatashield mounting medium (one drop) before mounting the coverslip

Mount max 2 coverslips per slides (easier to use in 780microscope)

After mounted use nail barnish to fix the sample and leave it dry.