Growth curve

The cells are to be plated in 12-wells plates
Organize plates so that the cell for a single point in the curve are together
Plate $2x10^4$ cells/well unless otherwise specified

> ***Plate the cells at the end of the afternoon and fix the first batch, « point zero » the next morning so that they have adhered but not started to proliferate***

Solutions
Crystal violet : 0,1% in PBS
Acetic acid : 10% in water, freshly prepared

<u>Protocol</u>
Plate preparation

- prepare plates, identify
- trypsinise and count the cells
- plate in about 2 mL media and put back in incubator

Fixation

- prepare a solution of 1% glutaraldehyde in PBS to a volume of 1 mL per well to be fixed
- aspire media
- wash 2 times with 2 mL of PBS
- fix 10 minutes with 1 mL of glutaraldehyde solution
- wash again 2 times, put a final 2 mL of PBS and keep plates, wrapped in cellophane, at 4° until all points are fixed

Staining

- when all the cells have been fixed, wash each well wih PBS, aspire and stain the cells with 1-2 mL of crystal violet solution for 30 min at RT°
- aspire staining solution (which you can reuse for many weeks)
- wash cell by immersion in water, 2-3 times until the wells are clear except for the cells…
- dry the plates at RT° or 37°
- dissolve in 1 mL acetic acid solution (put plates on rotary shaker)
- transfer 100 µL of each sample in a 96-well plate and mesure OD at 590 nm.

Results

- The point zero cells should be substracted as baseline in the calculations