Cell culture protocols

Dishes and plates:

	Surface	Minimum media	Maximum media
35 mm dishes or 6 wells plates	9.600 mm^2	1,5 mL	3 mL
6 cm dishes	2.827 mm^2	3 mL	6 mL
10 cm dishes	7.850 mm^2	7 mL	10-12 mL
15 cm dishes	17.867 mm^2	15 mL	35 mL

Media:

- **mEF media**: (Hek293T cells)
  - 225 mL DMEM Glutamax
  - 25 mL FBS (10%)
  - Filter sterilize

  } 250 mL total

Thawing out cells:

- Prepare enough media in a separate falcon tube and add PS (penicillin and streptomycin, 1:100).
- Prepare a 15 mL tube with 7 ml media (MEF, mES, KBM7…).
- Make sure pipets and everything is ready for use.
- Take the vial from -80ºC and thaw it rapidly at 37ºC (water bath) until there is a small clumb left.
- Take all the content of the vial and pipet it to the 15 mL tube.
- Centrifuge at 1100 rpm for 3 min and remove supernatant.
- Add 1 mL media and resuspend cells.
- Plate all cells and add required amount of media (depending on plate).

Cell passaging:

- Prepare enough media in a separate falcon tube and add PS (penicillin and streptomycin, 1:100).
- Remove media by aspirating
- Wash 2x with PBS and aspirate
- For a 10 cm dish add 1 mL Trypsin 0,05%/EDTA
- Incubate 2-3 min at 37ºC 0,5% CO_2
- Inactivate Trypsin adding 3x volume (Trypsin) of media
- Collect the cells + media + Trypsin
- Centrifuge at 1100 rpm 3 min
- Aspirate supernatant
- Add 1 mL media and resuspend
- Re-plate cell suspension in a desired concentration and add up to maximum amount of media per plate