**HEK293 cells storage, thawing and passaging**

**Splitting for Transfection and Passaging**

From a 10cm dish to 6 well plate

* Use a confluent 10cm dish. Warm the media (MEM + 10% FBS + Glutamax (5ml/500ml) + Pen/Strep (4-5ml per 500ml- MEM+++) and 1x st PBS.
* Once the media is warm, take out the 6well dish and fill 2ml of media in each well.
* Take out the confluent HEK cell plate from the incubator. Check under the microscope (90-100% confluency is preferred during splitting).
* Aspirate the media gently using a vacuum sucker. Gently pour a little of 1xPBS on one of the corners (see that you don’t lift off the cells) and rotate the plate so as to lift off any dead cells.
* Aspirate the PBS and add 10ml MEM+++ onto the plate. Lift the cells off the surface by using a 10ml pipette by pipetting up and down continuously to obtain a homogenous cell suspension (for HEK cells no trypsin is needed).
* Put this cell suspension into a new 10ml falcon.
* For achieving a confluency of 70-80% the next day (in a 6-well dish) add 1ml of the resuspended HEK cells into each well.
* 300-400 µl addition may give about 70% confluency after 2 days. Kindly titrate this according to your need and time of splitting and transfection.
* In case you want to plate out a 10 cm dish for further use, use 2ml of the resuspended cells and pour it onto a 10cm dish containing 10ml of fresh MEM +++ media (1:5 dilution).

**Storage**

* Aspirate the medium from the confluent TC plate(s).
* Gently pour a little of 1xPBS on one of the corners (see that you don’t lift off the cells) and rotate the plate so as to lift off any dead cells.
* Aspirate the PBS and add 10ml MEM+++ onto the plate. Lift the cells off the surface by using a 10ml pipette by pipetting up and down continuously to obtain a homogenous cell suspension (for HEK cells no trypsin is needed).
* Add about 10ml DMEM w/ 10% FBS and 1%P/S (DMEM++). Mix the cells well and centrifuge at 1200 rpm for 5 min.
* During this time, prepare and label the cryo preservation vials.
* Suck out the supernatant while leaving the cell pellet untouched. For each plate (whether you have pooled multiple plates or not), add 0.9ml of DMEM ++ and 0.1ml of st DMSO (either mixed previously or DMSO added later dropwise and gently mixed by inverting).
* Aliquot 1 ml in each of the cryovials and store them in the Cryo-storage box in -80 so as to slowly reduce the temperature of the vials. In absence of Cryo storage box, pack the vials in a Tupperware and store them in -20°C for 2-3 hrs, after which they could be moved in at -80°C for overnight.
* Store them the next day in Liquid Nitrogen for long term storage.

**Thawing HEK cells**

* Thaw the vial in the Bio safety cabinet (BSC).
* Prepare a 10cm dish with around 15 ml of prewarmed DMEM++ (or MEM ++).
* Gently aliquot the whole thawed tube into the dish.
* Swirl it gently and place it in the 37°C incubator.
* Check their health and confluency everyday.
* After two days, replace the old media with prewarmed fresh media.
* Split them once they are confluent.