HEK293 cells (Human embryonal kidney cells (HEK293, ATCC [Cat. No. CRC-1573]))

**Cell culture**
Medium 90% Minimum Essential Medium [Invitrogen/Gibco, Cat. No. 21099-022] with GlutaMAX [Invitrogen/Gibco, Cat. No. 35050-038] and 10% heat inactivated horse serum, 1 mM Sodium Pyruvate (Invitrogen/Gibco, Cat. No. 11360-039), 0.1 mM non-essential amino acids (Invitrogen/Gibco Cat. No. 11140-35)
Treatment (detachment) 0.5 mg/ml Trypsin; 0.2 mg/ml EDTA in PBS. Passage interval Cells should be passaged at 80-90% confluency. Seeding conditions 5x10^5 cells per 25 cm² flask.

**Protocol**
Culture conditions › The cells should be preferably passaged 2-3 days before nucleofection. HEK293 cells should not be used for nucleofection after passage number 20.
 › Cells should be nucleofected after reaching 80-90% confluency. Higher cell densities may cause lower nucleofection efficiencies.
Preparation of the › Add 0.5 ml Supplement to 2.25 ml Nucleofector™ Solution and mix gently. The Nucleofector™ Solution is now ready to use and is stable for 3 months at 4°C. **Note date of addition on the vial.**

- Try out to culture them with MEM, @ Glutamine, Pen-Strep, Amino-Acids, Na-Pyruvate.
  I split my HEK 2x/week 1:10, they are growing very fine!

**Culturing HEK 293 Cells**

**Reagents**
- Medium:
  500 ml Dulbecco’s Modified Eagle Medium (Gibco #41966-029)
  55 ml FCS (10 %)
  2.8 ml Gentamycin Solution (Sigma G-1272, 10 ml))
- Trypsin (10x) lyophilised (Gibco 25095-019)
- Versene 1:5000 (Gibco 15040-033, 100 ml)

  Take 2 bottles (2X100 ml) of Versene and pipet 10 ml of each bottle into the bottle with the lyophilised Trypsin to redissolve it. Then redistribute 10 ml of the Trypsin solution back into each Versene bottle.
- PBS
Procedures

Starting from a Kryotube:
Prewarm the medium at 37°C and fill two culture flasks (25 ml for 150 cm², 5 ml for 25 cm²). Rapidly thaw the cells (at 37°C) and distribute them in two concentrations in the flasks. Change the medium after 12 hrs or once the cells have attached.

Splitting Cells:
Aspirate the medium from the flask. Wash the cells carefully with PBS to remove residual medium. Add 1-2 ml of Trypsin Solution (equilibrated at RT) to the flask (150 cm²) and incubate at 37°C until cells have detached (1-2 minutes). Prepare a new flask with fresh medium. Block trypsinization by adding a few ml of medium. Take a fraction of the cell solution and inoculate the new flask. Typically, when splitting confluent HEK 293 cells in a 1:10 ratio, confluency is reached again after 2-3 days.

Maintaining HEK 293 Cells in Culture

HEK 293 cells should be grown in a monolayer, preferably in plastic petri dishes or flasks. Under optimum growth conditions (37°C, 5% CO₂), 293 cells double about every 36 hr. To maintain consistency, do not passage cells indefinitely. For best results, we recommend you use low passage 293 cells for transfection and titration procedures.

To prevent contamination, work with media and uninfected cells in a vertical laminar flow hood, using sterile technique. Keep this hood free of virus to prevent accidental infection of the stock cultures; ideally use another hood for all virus work. All virus-contaminated materials, including fluids, must be autoclaved or disinfected with 10% bleach or a chemical disinfectant before disposal.

Protocol Guidelines

1. To thaw 293 cells, place the vial of frozen cells in a 37°C water bath until just thawed. Sterilize the outside of the vial with 70% EtOH. For maximum viability upon plating, remove DMSO as follows:
   - Add 1 ml complete medium (prewarmed to 37°C). Transfer mixture to a 15-ml tube.
   - Add 5 ml complete medium and mix gently. Repeat. The final volume should be 12 ml.
   - Centrifuge at 125 x g for 10 min. Remove supernatant.
   - Gently resuspend cells in 10 ml complete medium: DMEM [or Minimum Essential Medium, α Modification (α-MEM)] supplemented with 100 units/ml penicillin G sodium, 100 µg/ml streptomycin, 4 mM L-glutamine, and 10% fetal bovine serum.
2. Transfer cells (in 10 ml of growth medium) to a 100-mm culture plate.
3. Cells should be split every 2–4 days when they reach 80–90% confluency. Cells should not be allowed to become overly confluent nor should they be seeded too sparsely.

4. Split the cells as follows. Remove the medium and wash the cells once with sterile PBS (containing no Ca²⁺ or Mg²⁺). Add 1–2 ml of trypsin-EDTA solution and treat for 1–3 min, just long enough to detach cells (do not expose cells to trypsin for extended periods). Then add 5–10 ml of complete growth medium (to stop trypsinization) and resuspend the cells gently but thoroughly. Transfer the desired number of cells to a 100-mm plate containing 10 ml of medium. Gently rock the plate to distribute cells.

Preparing Frozen Cultures of HEK 293 Cells

We recommend you prepare frozen aliquots of early passages of the HEK 293 cell line to ensure a renewable source of cells.

1. Expand the cell line in the desired number of flasks or plates.
2. When the desired number of flasks/plates have reached ~80% confluence, wash the cells once with sterile PBS (containing no Ca²⁺ or Mg²⁺), trypsinize, add 2–4 volumes complete medium to dilute trypsin, and harvest cells.
3. Count your cells and collect by centrifugation (~500 x g for 10 min).
4. Resuspend in 4°C Cell Freezing Medium at 1–2 x 10⁶ cells/ml.
5. Dispense 1 ml aliquots into labeled freezing vials and place in a cell freezing container (reduces temperature ~1°C/min) at −80°C overnight.
6. Alternatively, place the vials on ice or at −20°C for 1–2 hours, transfer to an insulated container (foam ice chest), and place container in a −80°C freezer for several hours to overnight.
7. Transfer vials to liquid nitrogen.
8. Two or more weeks later, confirm the viability of frozen stocks by starting a fresh culture.