HEK 293 cell, the Human Embryonic Kidney 293 cells was originally derived from human embryonic kidney cells grown in tissue culture. HEK293 cells are available from ATCC (www.atcc.org) , catalog# CRL-1573. It is easy to grow and transfect and have been widely used for cell biology research and also used by the biotechnology industry to produce therapeutic proteins and viruses for gene therapy.

Materials:

A. Media Components:

- DMEM, high glucose (Invitrogen Cat.#11965-118)
- Heat Inactivated Fetal Bovine Serum (HI FBS, Qualified, Invitrogen Cat.#16140-071)
- DMSO(Sigma-Aldrich Cat.#D2438)
- Dulbecco's Phosphate Buffered Saline(DPBS Invitrogen Cat.#14190-250)
- 0.05% Trypsin-EDTA 1X (Invitrogen Cat.#25300-054)
- Antibiotic-Antimycotic 100X(Anti-anti Invitrogen Cat.#15240)

A. Supplies and Equipment:

- 15ml/High Clarity Polypropylene Conical tubes(BD Falcon Cat.# REF 352096)
- 1.8 ml Cryo Tube Vials (NUNC, Cat.363401)
- Tissue Culture Flasks(BD Falcon, Cat.353136; 353132)
- Class II biological safety cabinet
- Hemacytometer
- Humidified 37^0C, 5% CO2 incubator
- Inverted microscope
- Cryo 1^oC freezing container(Mr.Frosty, Nalgene Cat.#5100-0001)

B.

C. Growth Media and Buffers:

2 Growth medium: DMEM, 10% HI-FBS, 1X Anti-anti

3 Freezing medium: 80% DMEM, 15% HI-FBS, 5% DMS

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Maintenance of HEK293 cell line

Thawing and Initial Culture Procedure

Rapidly thaw the cells by placing them at 37°C in a water bath with gentle agitation for 1–2 minutes.

Note: Freezing Medium may be yellow immediately after thawing. This does not affect cell viability if these instructions are followed.

- Decontaminate the vial by wiping it with 70% ethanol before opening in a class II biological safety cabinet.
- Slowly transfer the vial contents into 10ml of Growth Medium

in a sterile 15ml conical tube.

- Centrifuge the cells at 500 x *g* for 5 minutes at 18°C.
- Aspirate the supernatant and resuspend the cell pellet in 12ml of 37°C prewarmed Growth Medium.
- Transfer resuspended cells to a T75 flask, and culture cells.
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Propagation of Cells

- Cells should be maintained between 10% and 90% confluency in a 37°C, 5% CO2 tissue culture incubator. This typically will require passaging the culture twice a week. The approximate cell number for 100% confluence for this cell line in a T75 flask is 1 × 107 cells. Media formulations are provided in Section 1.C.
  Volumes listed are for propagation in a T75 flask.
- When cells have reached the appropriate density, aspirate the medium from the flask.
- Wash 1 time with 2ml 1X DPBS.
- Add 2ml of 37°C 0.05% Trypsin-EDTA. Evenly coat flask surface containing the cells. Trypsinize for 2 minutes.
- Using a microscope, verify that the cells have detached and clumps have completely dispersed.
- Stop trypsinization by adding 10 ml of growth medium.
- Transfer cell suspension to a conical tube. Determine cell number using a hemacytometer.
- Pellet cells at 500 × *g* for 5 minutes at 18°C.
- Aspirate the supernatant and resuspend cells in Growth Medium.
- Seed new flasks at appropriate cell density depending on the size of flask. For example, use 1 × 10^6 cells for a T75 flask.
- Place flasks in 5% CO2, 37°C incubator.

C.

D. Freezing Cells

- Grow cells to a density of 50% confluence. Replace Growth Medium + Anti-anti with Growth Medium (no Anti-anti) the day before harvest.
- Harvest cells as described above in Section 2.B.. After the cells have detached, spin cells down and resuspend them in Freezing Medium (Section 1.C.).
- Dispense 1.0ml per cryogenic vial.
- Place vials in an insulated container (i.e., Styrofoam® or Nalgene® Mr. Frosty, Cat. # 5100-0001) for slow cooling, and store overnight at –80°C.
- Transfer to liquid nitrogen tank or –140°C.