CULTURE OF HEK 293/293T CELLS

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BACKGROUND

- HEK 293/293T cells are derived from human embryonic kidney transformed with adenovirus 5 DNA
- HEK cells are easy to culture and transfect, and contain the SV40 large T-antigen, which allows for the episomal replication of plasmids containing the SV40 origin of replication

NOTES

- HEK cells are loosely adherent, and do not require digestion by trypsin/EDTA prior to being passaged.
- This protocol assumes that the cells are being cultured in T150 flasks

EQUIPMENT

- Equipment:
  - Tissue culture hood
  - 37°C water bath
- Materials:
  - DMEM supplemented with 10% FBS, 2mM L-glutamine and 100units/ml penicillin-streptomycin
  - PBS
  - 1X Trpsin
  - 1X Trypan blue
  - FBS
  - DMSO, tissue-culture grade
  - cyrovials

PROTOCOL

A. Thaw

- Preparatory Work:
  - Warm media to 37°C in water bath

1. Thaw frozen cells completely by hand or in a 37°C in water bath
2. Remove cells into 10ml fresh media in a centrifuge tube
3. Spin at 1000rpm for 5min
4. Replace supernatant with 20ml of fresh media
5. Transfer cell suspension into a flask

B. Passage

- Preparatory Work:
    - Warm media and PBS (and trypsin if needed) to 37°C in water bath

a) Without trypsin

1. Remove media from cells
2. Rinse cells with 5ml PBS
3. Wash off cells from bottom of flask or plate with 10ml of fresh media
4. Divide cells 1:5 (for passage after 48h) or 1:10 (for passage after 72h) into new flasks, adding fresh media for a total volume of 20ml in each flask

b) With trypsin (better for counting if it is necessary to seed cells)

1. Remove media from cells
2. Rinse cells with 5ml PBS
3. Add 5ml of trypsin, incubate 5min at 37°C
4. Add 5ml of complete media
5. Remove mix to a 50ml tube, spin down cells at 1500rpm for 5min
6. Re-suspend cells in 10ml fresh complete media
7. Divide cells 1:5 (for passage after 48h) or 1:10 (for passage after 72h) into new flasks, adding fresh media for a total volume of 20ml in each flask

C. Count

1. Continuing from Step 6 of Part B, above, remove 10ul of cells into a 1.5ml tube
2. Add 10ul of 1X Trypan blue, mix well
3. Count 10ul of mix on a hemocytometer

- Concluding Work:
    - Clean hemocytometer with alcohol before and after use.

D. Freeze

- Preparatory Work:
    - Warm media, PBS and 10% FBS to 37°C in water bath

1. Remove media from cells
2. Rinse cells with 5ml PBS
3. Wash off cells from bottom of flask with 10ml of fresh media and transfer to a centrifuge tube
4. Spin at 1000rpm for 5min
5. Re-suspend cells in 2.4ml FBS (do not leave cells sitting after this step for too long)
6. Add 300µl DMSO drop-wise
7. Mix by gently tapping
8. Transfer to cryovials, 1ml per vial