

Cell Culture Protocol for T-REx HEK293 cells

T-REx HEK293 (Invitrogen Catalog # R710-07); derived from HEK293 cells (ATCC CRL-1573):

Human Embryonic kidney cells; female embryonic kidney cells transformed with Adenovirus 5 DNA, and stably expressing Tetracycline repressor from the pcDNA6/TR plasmid.

Growth medium: DMEM (GIBCO # 11960) + 10% FBS + 2mM L-Glutamine + 100 units/ml penicillin + 100 micro-g/ml streptomycin (GIBCO # 15140-122) + 5 ug/ml Blasticidin (Invitrogen # R210-01).

Protocol for Thawing T-REx HEK293 Cells:

1. Take out the T-REx HEK293 stock vial from liquid nitrogen (we freeze at 2×10^6 cells per vial) and thaw it at room temperature.
2. Resuspend thawed cells in 10 ml growth media without Blasticidin and transfer cells into a 10 sq. cm. tissue culture dish. Cells are grown in a 37°C incubator at 5% CO₂.
3. Change the media to media with 5 ug/ml Blasticidin after 24 hours

Protocol for Subculturing of T-REx HEK293 Cells:

Change medium every 2 to 3 days, and split cultures when they reach 85% confluence (1×10^7 cells/10 sq. cm. dish)

1. Aspirate growth media from the tissue culture dish.
2. Add 5 mls of Trypsin (0.05%) with EDTA solution (GIBCO # 25300) and allow the cells to incubate in the 37°C incubator until cells detach.
3. Add 5 mls of fresh growth media and collect cells in a centrifuge tube.
4. Spin at 1500rpm for 5 minutes.
5. Aspirate supernatant and add fresh growth media
6. Transfer $1-5 \times 10^5$ viable cells/ml to a new culture vessel and place in a 37°C incubator at 5% CO₂

Note: We typically split cells at a ratio of 1:4 every 4 to 5 days using the above mentioned seeding conditions.

Freezing of T-REx HEK293 Cells:

Cells can be stored as a stock in liquid nitrogen at $2-5 \times 10^6$ cells/ml in growth medium containing 5% DMSO.

Special Note: These cells are used for inducible expression of exogenously expressed, Flag-tagged transcription factors, as well as for the analysis of endogenous factors. For induction, doxycycline is added to the cell culture medium at a final concentration of 1 ug/ml for 24 hours.