Propagation of TLA-HEK293T

Ordering Information

TLA-HEK293T can be ordered from Open Biosystems (Part of Thermo Fisher Scientific).

Name: HEK293T, human embryonic kidney
Catalog #: HCL4517

Notes: The human HEK293T cell line is a derivative of the HEK293 cell line, a human embryonic kidney cell line that was generated by transforming normal human embryonic kidney cells with adenovirus 5 DNA. It is an adherent cell line. The HEK293T cell line stably and constitutively expresses the SV40 large T antigen. It is for use in facilitating optimal lentivirus production.

Material List

1. DMEM, high glucose, pyruvate (Invitrogen, Cat.# 11995-065)
2. Fetal Bovine Serum (Cellgro, Cat# 35-016-CV)
3. L-Glutamine 200mM (100X), liquid (Invitrogen, Cat.# 25030-081)
4. Penicillin-Streptomycin (100X) (Invitrogen, Cat.# 15140-122)
5. Phosphate-Buffered Saline (PBS) (Invitrogen, Cat.# 10010-023)
6. 0.05% Trypsin-EDTA 1X (Invitrogen, Cat.#25300-054)
7. Recovery™ Cell Culture Freezing Medium (Invitrogen, Cat.# 12648-010)

Growth Medium for HEK 293T

DMEM
10% FBS
L-Glutamine (1x)
Penicillin-Streptomycin (1X)

Procedure

A. Thawing and starting cell culture

1. Rapidly thaw the cells in 37^{o}C water bath.
2. Add 1ml pre-warmed growth media into thawed cells dropwise. Swirl gently to mix.
3. Add the cell suspension to 9ml pre-warmed growth media dropwise in a 15ml conical tube . Swirl gently to mix.
4. Centrifuge at 700rpm for 5min.
5. Aspirate the media. Resuspend the cell pellet in 10ml pre-warmed media and transfer cells to a 100mm tissue culture dish.
6. Grow the cells in a 37^{o}C, 5% CO2 humidified incubator.

B. Sub-culturing

1. Check the cells every 2-3 days. Propagate cells until density reaches ~80% confluence
2. Aspirate the media and gently wash the plate once with 5ml pre-warmed PBS.
3. Add 1ml pre-warmed 0.05% Trypsin (1X) to dissociate cells for 1-3 minutes.
4. Stop trypsinization by adding 9ml of pre-warmed growth media
5. Passage cells to a ratio of 1:10 for general maintenance, or a smaller ratio as needed.

C. Freezing cells

1. Trypsinize one plate of 70~80% confluent cell. Count the cell number.
2. Collect the dissociated cell in 50ml conical and centrifuge at 700rpm for 5min.
3. Resuspend cell pellet in Recovery™ Cell Culture Freezing Media to a final density of 10^{6}

cells per ml.

4. Add 1ml cell suspension per cryogenic vial.
5. Place vials in an insulated container for slow cooling at –80°C overnight.
6. Transfer vials to liquid nitrogen tank or –140°C.