TECHNICAL MANUAL

HEK293T Cell Line

Production

The HEK293T cell line is supplied as a single vial containing ~ 1 × 10^6 frozen cells in 1 mL of freezing medium (includes 10% DMSO—Please review the SDS before handling here).

Shipping and storage

The Dharmacon™ HEK293T cell line is shipped on dry ice. While precautions have been taken to prevent CO_2 from entering the vial during shipment, it is suggested that upon receipt the cells be stored for two days or more in liquid nitrogen to allow any CO_2 to dissipate. When removing the vial from liquid nitrogen storage, leave at room temperature for approximately 30 seconds or longer to allow the liquid nitrogen to dissipate from the vial. **Note: Always wear protective eyewear and gloves when handling vials stored in liquid nitrogen.**

Quality control

The HEK293T cells have been tested for viability and verified to contain no Mycoplasma contamination.

Protocol for culturing HEK293T cells

Components for 10% DMSO in Hyclone™ HEK293T Culture Medium (Table 1).

Table 1. Dharmacon™ HEK293T culture medium

Reagent	Amount for 1 L of HEK293T Culture Medium	Final Concentration	HyClone Cat#
DMEM High Glucose, + Sodium Pyruvate (110 mg/L), no L-glutamine	870 mL	N/A	SH30285.02
L-glutamine (200 mM)	30 mL	6 mM	SH30034.01
Fetal Bovine Serum	100 mL	10%	SH30070.03
Penicillin (10,000 U/mL) /Streptomycin (10,000 µg/mL)	10 mL	100 U/mL/100 µg/mL	SV30010

Starting cells from frozen cell stock (thaw quickly)

1. Remove the HEK293T packaging cell line from liquid nitrogen and place in a 37 °C water bath for 2 minutes until nearly (~ 80%) thawed.
2. Remove the cells from the vial and add slowly into a 15 mL conical tube containing 10 mL pre-warmed Hyclone HEK293T Culture Medium.
3. Centrifuge for 3 minutes at 1000 × g to pellet cells and remove the supernatant.
4. Add 14 mL of Hyclone HEK293T Culture Medium and transfer cells to a T25 flask or a 100 mm culture dish.
5. Place the cells in the 37 °C incubator with 5% CO_2.
6. Gently replace medium after 24 hours with 14 mL HEK293T Culture Medium and continue to culture at 37 °C with 5% CO_2. Cells should be ready for passage or expansion to a T175 flask in ~ 2-3 days. See images in FAQ section for examples.

HEK293T cells detach easily from the culture dish surface, therefore handle the cells gently when replacing the culture medium or during washing.

Cell maintenance

1. Fresh HEK293T Culture Medium should be added to the cells every 3 days or as required by the growth rate of the cells.
2. HEK293T cells should always be treated very gently as they detach easily from the plate.
3. Add an appropriate volume of pre-warmed HEK293T Culture Medium to the cells. You may first need to rinse the cells with PBS (HyClone Cat #SH30028.03) or medium prior to feeding the cells.

This is typically done if there is a high degree of dead cells or debris in the culture, as would be expected after a thaw.

4. Return the cells to the 37 °C incubator with 5% CO_2.

Sub-culturing/passaging of cells

Passage cells at least 3 times before using them in desired application. For lentiviral packaging, refer to Dharmacon Trans-Lentiviral Packaging technical manual on our web site.

1. HEK293T cells are typically passaged when 80% confluent to a ratio of 1:10 to 1:20 for general maintenance.

Cells can be passaged using a smaller ratio but will then reach confluency quicker and will need to be passaged more frequently (for example 1:5). We recommend 4:5 or 1:1 ratio for viral packaging applications.

2. Carefully aspirate the growth medium from the cells. This is best done by tilting the flask or plate and removing the medium without touching the cell surface.
3. Gently wash cells with PBS.
4. Trypsinize the cells (Table 2) with appropriate amount of trypsin (HyClone Cat #SH30042.01). Place plate in the 37 °C incubator for ~ 2 minutes for cells to release from the plate.
5. Add appropriate volume for cell culture vessel of HEK293T Culture Medium to resuspend cells and inactivate the trypsin.
6. Pipette cells up and down ~ 5 times with a 10 mL pipette to get a single cell suspension, while avoiding frothing of medium.
7. Plate cells into new sterile flasks or plates containing HEK293T Culture Medium (Table 3). Place the cells at 37 °C with 5% CO_2.

Table 2. Trypsinization and resuspension volumes for routinely used vessels.

Cell culture vessel	PBS wash (mL)	Trypsin (mL)	Resuspension cell growth (mL)	Recommended volume in new flask (mL)
T-25 or 100 mm	2.5	1	5-10	5-10
T-150	10	2	10	30-40
T-175	10	2	10	35-50

Table 3. Flask and plate surface areas and recommended volumes.

Flask type	Growth area per well (cm^2)	Volume growth per well
T-175	175	35-50
T-150	150	30-40
100 mm dish	55	10
T-25	25	10
6-well	9.5	3
12-well	4	2
24-well	2	1
48-well	1	0.5
96-well	0.32	0.1
8-well chamber	0.8	0.4

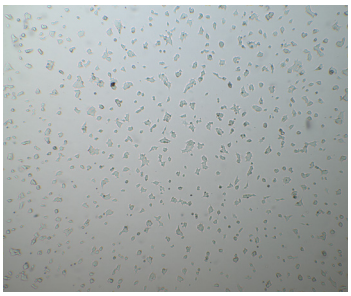
FAQs

Can other sources of HEK293T cells be used for packaging?

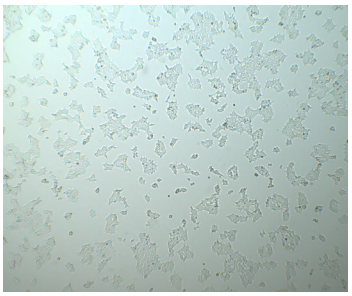
Yes, any standard HEK293T cells can be used for packaging your lentivirus. It is recommended to use a cell line that stably expresses the SV40 large T antigen to facilitate the production of high viral titers. Using a cell line without the SV40 large T antigen will result in lower titers.

I am seeing a large amount of cell death after I plate my cells. When following the recommendations above, what can I expect to see?

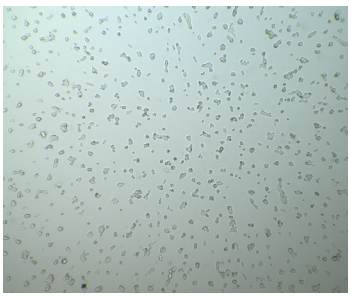
24 hours, before wash (100 mm dish)



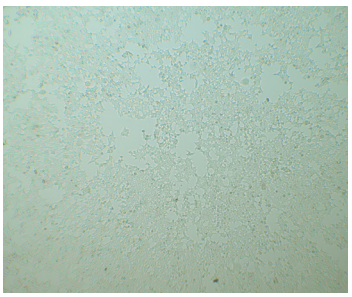
24 hours, after wash (~ 70% recovery)



48 hours



96 hours



If you have any questions, contact

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V2-0618