Oct 17, 2025

Tissue culture protocol for HEK293 cells

In 1 collection

DOI
https://dx.doi.org/10.17504/protocols.io.bp2l6zy7dgqe/v1

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Protocol Citation: Susanne Herbst, Patrick Lewis 2025. Tissue culture protocol for HEK293 cells. **protocols.io https://dx.doi.org/10.17504/protocols.io.bp2l6zy7dgqe/v1**

Protocol status: Working
We use this protocol and it's working

Created: October 17, 2025

Last Modified: October 17, 2025

Protocol Integer ID: 230072

Keywords: ASAPCRN, Tissue culture, hek293 cells lewis lab tissue culture protocol, hek293 cell, tissue culture protocol

Funders Acknowledgements:
ASAP

Abstract

Lewis lab tissue culture protocol for HEK293 cells.

Materials

- DMEM (31966047, Gibco)
- heat-inactivated FCS
- Trypsin-EDTA (25300054, Gibco)
- PBS
- DMSO

Troubleshooting

Subculturing

1 Remove the medium and add 5 ml of PBS to 75 cm^2 flask.

2 Remove PBS and add 3 ml of Trypsin-EDTA (25300054, Gibco).

3 Place flask back in incubator for 2 min.

4 Check if cells have detached, then add 7 ml of complete medium.

5 Aliquot cell suspension into new flask at a ratio of 1:3 to 1:5.

6 Incubate the culture at 37°C, 5% CO2 in a suitable incubator.

7 Subculture every 2 to 3 days.

Thawing

8 Thaw the vial by gentle agitation in a 37°C water bath. To reduce the possibility of contamination, keep the O-ring and cap out of the water. Thawing should be rapid (approximately 2 minutes).

9 Remove the vial from the water bath as soon as the contents are thawed, and decontaminate by dipping in or spraying with 70% ethanol.

10 Centrifuge the cell suspension at approximately 300 x g for 5 minutes. Discard the supernatant and resuspend the cell pellet in an appropriate amount of fresh growth medium and transfer the cells to an appropriate size vessel.

11 Incubate the culture at 37°C, 5% CO2 in a suitable incubator.

Freezing

12 Freeze cell suspension in complete growth medium supplemented with 10 % (v/v) DMSO at 1 to 5 × 10^6 cells/ml.