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Tissue culture protocol for HEK293 cells

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Susanne Herbst^{1,2}, Patrick Lewis^{1,2}

¹RVC;

²The Michael J. Fox Foundation for Parkinson's Research (MJFF) and the Aligning Science Across Parkinson's (ASAP) Initiative



Susanne Herbst

RVC

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Protocol status: Working

We use this protocol and it's working

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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 cm² 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.