Sep 06, 2022

Culture and transfection of HEK293T cells

DOI
https://dx.doi.org/10.17504/protocols.io.eq2lynkzpvx9/v1

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DOI: https://dx.doi.org/10.17504/protocols.io.eq2lynkzpvx9/v1

External link: https://doi.org/10.7554/eLife.79208

Protocol Citation: Hanqin Li, Dirk Hockemeyer 2022. Culture and transfection of HEK293T cells. **protocols.io** https://dx.doi.org/10.17504/protocols.io.eq2lynkzpvx9/v1

Manuscript citation:
Hanqin Li, Oriol Busquets, Yogendra Verma, Khaja Mohieddin Syed, Nitzan Kutnowski, Gabriella R Pangilinan, Luke A Gilbert, Helen S Bateup, Donald C Rio, Dirk Hockemeyer, Frank Soldner (2022) Highly efficient generation of isogenic pluripotent stem cell models using prime editing eLife 11:e79208

https://doi.org/10.7554/eLife.79208

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

Created: July 23, 2022

Last Modified: December 13, 2024

Protocol Integer ID: 67395

Keywords: ASAPCRN, transfection, cell, lipofectamine

Funders Acknowledgements:
Aligning Science Across Parkinson's
Grant ID: ASAP-000486

Abstract

This protocol describes a standard procedure culturing and transfecting HEK293T cells

Protocol overview:
A. Culturing HEK293T cells
B. Transfection of HEK293T cells with Lipofectamine 2000

Materials

	A	B	C
	Item	**Vendor**	**Catalog #**
	DMEM, high glucose	Thermo Fisher	11965118
	DPBS w/o Calcium and magnesium	Corning	MT21031CV
	Fetal Bovine Serum (FBS)	Corning	35-011-CV
	L-Glutamine	Sigma	G8540
	MEM Non-Essential Amino Acids (100X)	Thermo Fisher	11140050
	0.25% Trypsin with EDTA	Thermo Fisher	25200114
	Opti-MEM	Thermo Fisher	31985062
	Sodium Pyruvate 100 mM	Thermo Fisher	11360070
	Lipofectamine 2000	Thermo Fisher	11668019

A. Culturing HEK293T cells

1m

1 HEK293T cells are cultured in HEK293T medium in 10 cm dishes.

1.1 **HEK293T Medium**

	A	B
	DMEM, high glucose	385 ml
	Fetal Bovine Serum (FBS)	50 ml
	L-Glutamine (100X)	5 ml
	MEM Non-Essential Amino Acids (100X)	5 ml
	Sodium Pyruvate 100 mM	5 ml

Final volume: 500 mL

2 Passage cells when the culture reaches 80% confluency.

3 Remove medium

4 Wash once with 5 ml DPBS

5 Add 1 ml 0.25% Trypsin with EDTA, tilt and shake the dish so that the Trypsin covers the entire dish.

6 Incubate at 37 °C for 00:01:00

1m

7 Add 5 ml fresh HEK293T medium to inactivate Trypsin

8 Pipet 10 times to dissociate the cells and mix

9 Transfer 1 ml of cell suspension to a new dish pre-added with 9 ml HEK293T medium. Shake to mix well.This is a 1:6 splitting.

10 HEK293T cells usually needs to be passaged every 2 days.

B. Transfection of HEK293T cells with lipofectamine 2000

45m

11 One day before transfection, dissociate HEK293T cells with Trypsin as described above

12 Seed 250,000 cells/1 well of 12-well plate

13 Change to fresh medium 1 h before transfection

14 Label two micro centrifuge tubes as I and II

15 In micro centrifuge tube I, add 125 µl Opti-MEM and 6 µl Lipofectamine 2000. Mix by gently pipetting 3 times. Incubate at Room temperature for 00:05:00 . 5m

16 While waiting, in micro centrifuge tube II, add 125 µl Opti-MEM and 250 ng total plasmid. Mix by pipetting.

17 Mix tube I and II by gently pipetting 3 times. Incubate at Room temperature for 00:20:00 . 20m

18 Mix one time by gently pipetting. Transfer all transfection reagents into one well of a 12-well plate, drop-wise.

19 After adding the reagent to all wells, shake the plate to mix.

20 Culture in 37 °C incubator Overnight . 20m

21 Change to fresh medium and culture for another 2 days. Collect samples or passage once if longer culturing is needed.