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Culture and transfection of HEK293T cells

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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

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PROTOCOL CITATION

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MATERIALS TEXT

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.

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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

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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.

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