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Transfection of HEK cells with Lipofectamine 3000

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Protocol status: Working

We use this protocol and it's working

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Abstract

how to transfect HEK cells with lipofectamine 3000

Materials

Lipofectamine 3000 - [Lipofectamine 3000](#)

Opti-MEM reduced serum medium - [Opti-MEM reduced serum medium](#)

Cell preparation - Day 0

- 1 Plate HEK cells in 24 well plate: 100K per well

Day 1

- 2 Check cells → less than ~60-70% confluent wait another day, if 60-70% confluent can begin lipofectamine transfection

Lipofectamine Transfection

15m

- 3 Be sure to measure your DNA concentration before beginning the transfection - as you want a concentration of 0.5-5 ug/ul. Dilute accordingly
- 4 Calculate how many wells should be transfected. The below graph is the amount of materials needed to transfect 1 well in a 24 well plate. Multiply the materials accordingly depending on how many wells needed.
- 5

	A	B	C	D	
1	Step	Tube	Complexation components	Amount per well (24-well)	
2	1	Tube 1	Opti-MEM I medium	25 µL	
3			Lipofectamine 3000 reagent	1.5 µL	
4			Opti-MEM I medium	25 µL	
5	2	Tube 2	DNA amount (DNA concentration should be 0.5-5 µg/µL)	2.5 µL	
6			P3000™ reagent	1 µL	
7	3		Add tube 2 solution to tube 1 and mix well		
8	4		Incubate mixture from step 3 at room temperature for 10-15 min		
9	5		Add 50 µL of complex from step 4 to cells; gently swirl plate to ensure homogeneous distribution of complex to the entire well		

Lipofectamine Transfection components

- 6 Prepare 2 tubes per plasmid. One tube will have Opti-MEM reduced serum medium medium with the lipofectamine 3000 reagent and the other will have Opti-MEM reduced serum medium with the plasmid and P3000 reagent (see above graph)
- 7 Add the solution from tube 2 into tube 1 and mix well
- 8 Incubate the mixture for 10-15 minutes at RT

15m



- 9 Add 50 ul of the mixture from step 7 to each well. Gently swirl plates to ensure homogenous distribution of complex to the entire well