

08. (August) 2019

Project: iGEM_Munich2019 Shared Project

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SATURDAY, 24/8/2019

Alejandro:

reuptake assay - fluc. recipient cells

- Time: 10:30 a.m.; 24 h after transfection
- medium was discarded and cells were resuspended in 1 mL cold PBS (16 times up and down)
- 52 µL suspension were used for the fluc assay and a 0, 10, 20, 30, 40, 50 fmol HiBit standard curve in PBS was used
- 5 min incubation time at 300 min⁻¹

Fluc assay for reuptake assay, 24/08/19												
	1	2	3	4	5	6	7	8	9	10	11	12
A	HiBit standard curve 0-50											
B												
C												
D												
E	1: only Gag *		2: only fluc *		3: MCP VLPs *		4: L7Ae VLPs *					
F												
G												
H												

- * 2 wells with supernatant from origin cells 1, 2 wells with supernatant from origin cells 2, 2 wells with supernatant from origin cells 3

cell culture

T75 flasks transfection for qPCR and Purification

○ Important guidelines

Make DNA-Lipofectamine™ 3000 complexes in serum-free medium such as Opti-MEM™ Reduced Serum Medium and add directly to cells in culture medium, in the presence or absence of serum/antibiotic.

It is not necessary to remove complexes or change/add medium after transfection.

The amount of Lipofectamine™ 3000 Reagent for successful transfection varies. Start any new transfection by testing the recommended two concentrations of Lipofectamine™ 3000 Reagent to determine an optimum amount

○ materials:

- Plasmid DNA (0.5-5µg/µL stock)
- Opti-MEM Reduced Serum Medium
- Microcentrifuge tubes
- Lipofectamine 3000 reagent
- Cells (e.g. HEK 293 T)

- both flasks were at about 50-60 % confluent
- medium over the cells was removed and 15 mL new medium was added
- time transfection finished: 13:30 p.m.
- T75 flask VLPs-MCP

T75 flask VLPs-MCP				
	V8	V11	V14	V27
1	8 µg	4 µg	4 µg	4 µg


- T75 flask Exosomes-L7Ae

T75 flask Exosomes-L7Ae		
	E10	V15
1	12 µg	8 µg

- Transfectionscheme

transfectionmix T75		
	A	B
1	DNA per flask	20 µg
2	P3000 Reagent per flask	40 µL
3	Lipofectamine 3000 reagent per flask	30 µL
4	OptiMEM per flask	2 x 750 µL

- Transfect cells according to the following table. Use the indicated volume of DNA and P3000™ Reagent with each of the two volumes of Lipofectamine™ 3000 (when performing optimization). Each reaction mix volume is for one well and accounts for pipetting variations. Scale volumes proportionally for additional wells.



Timeline		Steps
Day 0		Seed cells to be 70–90% confluent at transfection
Day 1		Dilute Lipofectamine™ 3000 Reagent in Opti-MEM® Medium (2 tubes) – Mix well
Day 1		Prepare master mix of DNA by diluting DNA in Opti-MEM® Medium, then add P3000® Reagent – Mix well
Day 1		Add Diluted DNA to each tube of Diluted Lipofectamine™ 3000 Reagent (1:1 ratio)
Day 1		Incubate
Day 1		Add DNA-lipid complex to cells
Day 4		Visualize/analyze transfected cells

Procedure Details (Two Reaction Optimization)				
Component	8-well	24-well	48-well	6-well
Adherent cells	1.4 × 10 ⁶	0.5–2 × 10 ⁶	0.25–1 × 10 ⁶	
Opti-MEM® Medium	5 µL × 2	25 µL × 2	125 µL × 2	
Lipofectamine™ 3000 Reagent	0.15 and 0.3 µL	0.75 and 1.5 µL	3.75 and 7.5 µL	
Opti-MEM® Medium	10 µL	50 µL	250 µL	
DNA (0.5–5 µg/µL)	0.2 µg	1 µg	5 µg	
P3000® Reagent (2 µL/µg DNA)	0.4 µL	2 µL	10 µL	
Diluted DNA (with P3000® Reagent)	5 µL	25 µL	125 µL	
Diluted Lipofectamine™ 3000 Reagent	5 µL	25 µL	125 µL	
Incubate for 10–15 minutes at room temperature.				
Component (per well)	8-well	24-well	48-well	6-well
DNA-lipid complex	10 µL	50 µL	250 µL	
DNA amount	100 ng	500 ng	2500 ng	
P3000® Reagent	0.2 µL	1 µL	5 µL	
Lipofectamine™ 3000 Reagent used	0.15 and 0.3 µL	0.75 and 1.5 µL	3.75 and 7.5 µL	
Incubate cells for 2–4 days at 37°C. Then, analyze transfected cells.				