08. (August) 2019

Project: iGEM_Munich2019 Shared Project
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TUESDAY, 6/8/2019

Thess

cell culture: transfection for VLP Purification

- 550 µL medium were exchanged before transfection
- all conditions were seeded as duplicates

transfection scheme Purification assay 30/07/2019

	condition	V8	V27	V28
1	W1	250 ng	250 ng	-
2	W2	167 ng	333 ng	-
3	W3	333 ng	167 ng	-
4	W4	250 ng	-	250 ng
5	W5	167 ng	-	333 ng
6	W6	333 ng	-	167 ng

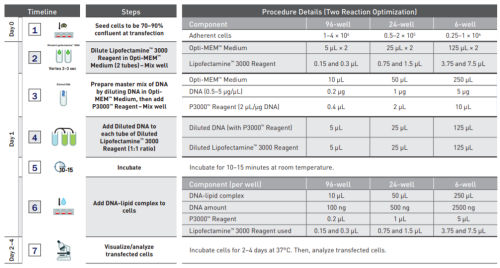
- Transfection:

Transfectionmix 24-well plate 30/0…

	A	B
1	DNA per well	500 ng
2	P3000 Reagent per well	1 µL
3	Lipofectamine 3000 reagent per well	0.75 µL
4	OptiMEM per well	2 x 25 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.

image.png



Timeline

Steps

Seed cells to be 70–90% confluent at transfection

Dilute Lipofectamine™ 3000 Reagent in Opti-MEM™ Medium (2 tubes) – Mix well

Prepare master mix of DNA by diluting DNA in Opti-MEM™ Medium, then add P3000™ Reagent – Mix well

Add Diluted DNA to each tube of Diluted Lipofectamine™ 3000 Reagent (1:1 ratio)

Incubate

Add DNA-lipid complex to cells

Visualize/analyze transfected cells

Procedure Details (Two Reaction Optimization)

Component	96-well	24-well	6-well
Adherent cells	1–4 × 10⁴	0.5–2 × 10⁵	0.25–1 × 10⁶
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)	96-well	24-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.

Anja

cell culture: transfection for hArc

- 550 µL medium were exchanged before transfection
- all conditions were seeded as duplicates

transfection scheme hArc assay 06/08/2019

	V8	V25	V30
1	-	-	500 ng
2	500 ng	-	-
3	-	500 ng	-

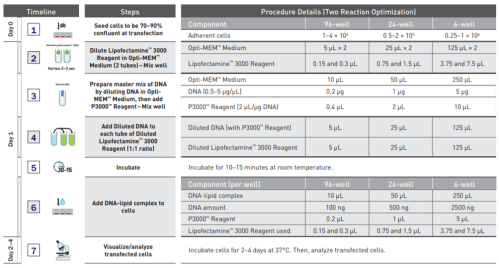
- Transfection:

Transfectionmix 24-well plate 06/0…

	A	B
1	DNA per well	500 ng
2	P3000 Reagent per well	1 µL
3	Lipofectamine 3000 reagent per well	0.75 µL
4	OptiMEM per well	2 x 25 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.

image.png



Timeline	Steps
Day 0 — 1	Seed cells to be 70–90% confluent at transfection
2	Dilute Lipofectamine™ 3000 Reagent in Opti-MEM™ Medium (2 tubes)—Mix well
3	Prepare master mix of DNA by diluting DNA in Opti-MEM™ Medium, then add P3000™ Reagent—Mix well
Day 1 — 4	Add Diluted DNA to each tube of Diluted Lipofectamine™ 3000 Reagent (1:1 ratio)
5 — 10-15	Incubate
6	Add DNA–lipid complex to cells
Day 2–4 — 7	Visualize/analyze transfected cells

Procedure Details (Two Reaction Optimization)

Component	96-well	24-well	6-well
Adherent cells	1–4 × 10^4	0.5–2 × 10^5	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)	96-well	24-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.