

08. (August) 2019

Project: iGEM_Munich2019 Shared Project

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Joshi

Cell culture: Transfection of HEK293T (24-well plate, according to Mamallian cell transfection with Lipofectamine 3000)

A : 12h

B : 24h

C : 48h

D : 72h

18082019_Transfection_EXOs_Origin_cells						
	1	2	3	4	5	6
A	AL, MeL	AL, MeL	AM, MeM	AM, MeM	MeL	M
B	AL, MeL	AL, MeL	AM, MeM	AM, MeM	MeL	M
C	AL, MeL	AL, MeL	AM, MeM	AM, MeM	MeL	M
D	AL, MeL	AL, MeL	AM, MeM	AM, MeM	MeL	M

Cell culture: HEK293T seeding (24-well plate, 150.000cells/well, 800ul full DMEM/well)

Cell culture: Medium transfer 12h Origins --> Recipients

Cell culture: Cell washing*: take off 700ul old medium, wash with 700ul DPBS, gently shake, take-off, repeat once, add 700ul fresh full DMEM

*perform separately, not for multiple wells at once and put the plate in the incubator in between to prevent cells from being stressed too much

Sarah

His-purification

Transfected:

- 3ng of His-AM, 2ng of MeM
- 3ng of AM, 2ng of MeM
- $V_f = 3\text{ml}$

Gravity flow instead of centrifugation (HisPur NiNTA resin beads)

- equilibrate beads with PBS
- incubate 1h at 4°C
- wash with freshly prepared buffer: 3CV (25mM imidazole, PBS pH=7.4, approx. 4.5ml)
- elute with freshly prepared buffer 2CV (250mM imidazole, PBS pH=7.4, approx. 3ml)
- perform HiBiT Assay (according to VLPs / Exosomes harvesting & HiBiT assay from 96-well plates: step 9)