08. (August) 2019

Project: iGEM_Munich2019 Shared Project
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WEDNESDAY, 28/8/2019

RT Exosomes Kit purified

- A5
- 5x RT and 5 x no RT
- only supernatant

RT EXO Kit and VLP

- samples from T75 flask
- 2x VLP SN
- 1x VLP cell
- 1x Exo SN Kit
- + same for no RT

Finished VLP-WB against MCP after protocol
4: gag-hibit-cc9-cc10-MCP
5: gag-hibit-cc9-cc11-MCP
6: gag-hibit-cc9-cc10-L7Ae

Workflow: RNA isolation out of exosomes and VLPs out of T75 flask including cell samples
Exosomes centrifugated and Pellet (visible) is resuspended in 1 ml PBS, addition of 10 µl DNase 1 and 100 µl DNase 1 buffer.
VLPs: 16 ml were taken out of flask, centrifugated at 2,000 x g for 10 min at 4 °C and 15 ml were recovered, 3 ml are taken away for purification, addition of 600 µl DNase 1 buffer and 60 µl DNase 1 were given to 6 ml VLP supernatant

Cells of VLPs: 4 ml of CLB were given to the flask and incubated for 5 min at 4 °C, pippet up and down to lyse the cells, spin down the cells at 3,000 x g for 10 min at 4 °C, recover the remaining 3.2 ml and separate in two Eppis, and 16 µl DNase 1 and 100 µl DNase buffer

Stored at 37 °C for one hour
Exosomes and VLPs are lysed in 1 % Triton for 10 min at 60 °C (Volume 1:!, so 6 ml VLP were added to 6 ml 1% Triton)

RNA Isolation

One Falcon of VLP was purified via RNeasy purification kit and the other via TrIzol. For TRIzol, the falcons are devided into two and the same volume of TRIzol is added (6 ml sample: 6 ml TRIzol) 1.8 ml
Then 12,000 x g for 15 min at 4 °C and normal protocol. When precipitation in 75 % EtOH overnight at 4 °C, the VLP SN samples were pooled again into two Eppis.
For exosomes SN and VLP cell: normal TRIzol RNA isolation kit, samples

Nanodrop Measurment 28.08

	Sample	concentration ng/µl	260/280	260/230
1	Exo SN Kit	2563	1.53	0.75
2	VLP cell	4266.4	1.45	1.20
3	VLP SN 1	3644.4	1.57	1.03
4	VLP SN 2	4034.9	1.57	1.13

qPCR 28/29.08.19