

# 07. (July) 2019

**Project:** iGEM\_Munich2019 Shared Project

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THURSDAY, 4/7/2019

## His Tag Purification of VLP & Exosome

- protocol like 06.06.19
- samples split in three tubes with equal volume of 200µl (3x VLP & 3x Exosomes)

## Lysis of VLPs & Exosomes

- one sample (VLP & Exo) incubated on ice
- second sample mixed with 1% Triton 1:1 (-> 200µl), incubation 10min at 60°C
- third sample incubated at 90°C for 10min, followed by sonication in water bath 3x5min

## RNA isolation

- samples were split in half
- first half -> Phenol-Chloroform extraction
- second half -> RNeasy Mini Kit protocol

Table1

	Sample	Concentration (ng/µl)	260/280	260/230
1	1. Ex Kit	2.4	0.92	0.03
2	2. Ex Triton Kit	6.5	1.09	1.09
3	3. Ex Ultrasonication Kit	37.3	1.45	0.23
4	1. VLP Kit	too low to detect		
5	2. VLP Triton Kit	too low to detect		
6	3. VLP Ultrasonication Kit	too low to detect		

## Phenol-/Chloroform Extraction

1. add 300µl phenol/chloroform/IAA mix to 100µl of exosomes & shake to mix phases
2. wait for 5min
3. Centrifuge at 12 000xg for 5min at room temperature
4. remove aqueous phase & add to new Eppi (don't transfer any phenol!)
5. add 300µl phenol/chloroform mix to organic phase of first isolation & vortex
6. Centrifuge at 12 000xg for 5min
7. remove aqueous phase & add to same eppi as in step 4
8. add equal volume of chloroform to mix & vortex
9. Centrifuge at 12 000xg for 5min
10. transfer water phase to new eppi
11. add 5 Vol ice cold 95% ethanol & mix well, store for 20min at room temperature

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12. spin 20min at full speed at 4°C, remove supernatant without disturbing pellet
13. air dry pellet (max 2min)
14. resuspend pellet with 20µl nuclease-free water

Exosomes were diluted 1:100, VLP diluted 1:500 in nucfree H2O

Table2				
	Sample	Concentration n ng/µl	260/280	260/230
1	1. VLP P/C	24.5	1.50	1.98
2	2. VLP Triton P/C	33	1.52	2.01
3	3. VLP Ultrasonication P/C	35.5	1.52	1.93
4	1. Exo P/C	4.1	1.49	2.18
5	2. Exo Triton P/C	8.4	1.48	2.23
6	3. Exo Ultrasonication P/C	8.6	1.58	2.11

#### Aliquoting the Antibodies CD63 & p24

CD63: 100µg, 1.0mg/ml

0.1/1 = 0.1ml = 100µl total volume

pro WB: 2µg/ml -> 0.002mg/ml : 1.0mg/l \* 5ml = 7.6µl

15µl Aliquote für je 2 WBs

p24: 100µg, 1.319mg/ml

0.1/1.31 = 0.076 = 76µl total volume

2µg/ml -> 0.002mg/ml : 1.31mg/ml \* 5ml = 10µl

20µl Aliquote für je 2 WBs