

05. (May) 2019

Project: iGEM_Munich2019 Shared Project

Authors: Theresa Keil

THURSDAY, 2/5/2019

Exosome Precipitation

Stock Solutions

- PEG 8,000 (-)
 - 10 ml ddH₂O
 - 20 % PEG 8000 (2.014 g)
 - 2.5 M NaCl (1.463 g)
- PEG 8,000 (+)
 - 5 ml PEG 8000 (-)
 - 0.25 % Protamine (2.5 g)
- PEG 35,000 (-)
 - 10 ml ddH₂O
 - 20 % PEG 35,000 (2.002 g)
 - 2.5 M NaCl (1.463 g)
- PEG 35,000 (+)
 - 5 ml PEG 35,000 (-)
 - 0.25 % Protamine (2.5 g)

Stir at 500 rpm, 35-40 °C, till solution is clear

Filter with 0.22 µm filter

Conditioned medium was collected and centrifuged at 3,500 x g (7,200 rpm eppendorf mini spin) for 20 min at RT

Transferred supernatant into new eppi and the centrifugation step was repeated

! At the different centrifugation steps no pellet could be seen !

Aliquots of 200 µl times 4 were made from the supernatant

200 µl of each stock solution were mixed with one aliquot supernatant

Samples					
	A	B	C	D	E
1	Plasmid	8000 (-)	8000 (+)	35000 (-)	35000 (+)
2	CD63-L7Ae (462)	462	462	462	462
3	nluc-C/D-box (465)	465	465	465	465
4	462+465	2+5	2+5	2+5	2+5
5	Booster (B)	B	B	B	B
6	Booster+462+465	B+	B+	B+	B+
7	PBS	PBS	PBS	PBS	PBS

Samples were incubated over night at 4°C