05. (May) 2019

Project: iGEM_Munich2019 Shared Project
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THURSDAY, 16/5/2019

SDS-Page exosomes from 02.05.19
samples were diluted 1:1 with MiliQ
SDS-PAGE according to protocol SDS-PAGE and Coomassie Staining

SDS-PAGE scheme

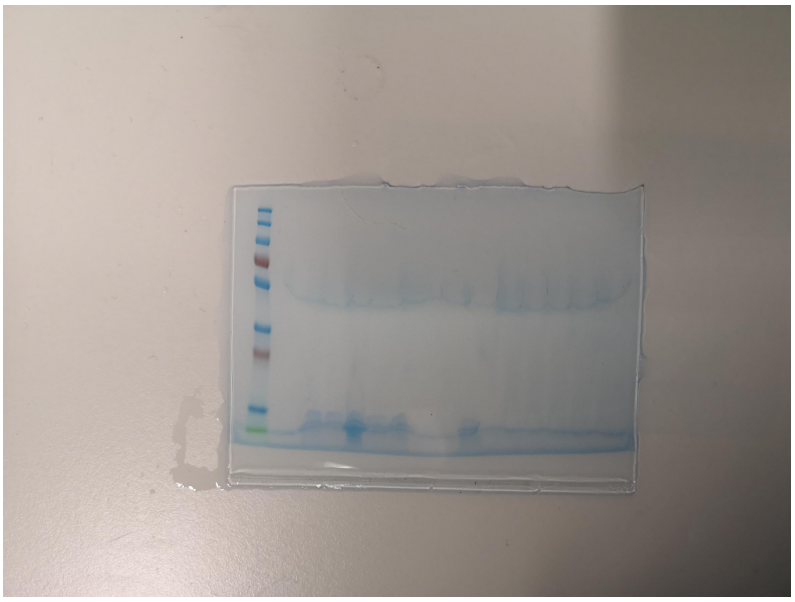
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	markter		Booster	PBS	462	465	2+5	B+		Booster	PBS	462	465	2+5	B+
2			with Protamine							without Protamine					

PEG 35,000 gel : B+ with protamine in pocket 9 instead of 8 because pocket 8 was broken when comb was removed

the empty pockets were loaded with 15 µl Laemmli (2x)

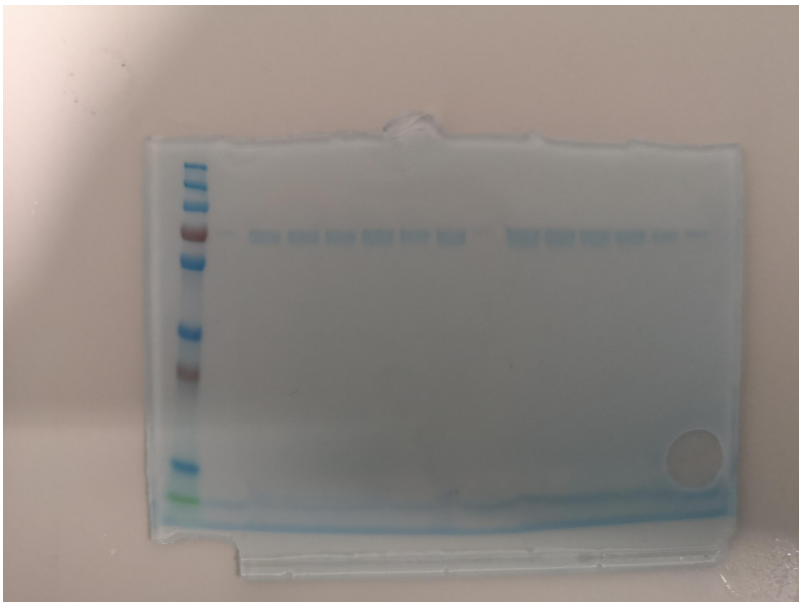
Run was normal this time -> make sure that enough buffer is between the gels!
Coomassie Staining according to protocol: staining 10 min, destaining 3.5 h
Incubation over night at 4 °C in Fixative solution (protocol: Silver Staining)

SDS_16.05_35000.jpg



PEG 35,000 seems to interfere with gel run, try to remove PEG better the next time before run

SDS_16.05_8000.jpg



PEG 8000: not the same problem as PEG 35,000,

one high band: expected more than one band, because of the different membrane proteins but could be over-expressed CD63 fusion protein with glycosilation
Note: try to find out how glycosylation changes gel run

Exosome Precipitation

1. Centrifuged supernatant at 1,000 x g for 20 min, removed the supernatant as good as possible (no pellet could be seen)
2. Filtered the supernatant with 0.22 µm filter
3. Prepared PEG/protamine solutions
    a. PEG 8,000: 20 % PEG 8,000 (2.0 g) + 75 mM NaCl (0.04383 g)
    b. PEG 8,000 + Protamine: added to 1.5 ml of PEG 8,000 solution 0.75 mg
    c. PEG 35,000: 20 % PEG 35,000 (2.0 g) + 75 mM NaCl (0.04383 g)
    d. PEG 35,000 + Protamine: added to 1.5 ml of PEG 35,000 solution 0.75 mg
4. Made 150 µl aliquots of the supernatant for a and c and 100 µl aliquots for of the supernatant for b and d
5. Added one volume of the corresponding PEG solution to the aliquots
6. Incubated samples over night at 4 °C