07. (July) 2019

Project: iGEM_Munich2019 Shared Project
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TUESDAY, 16/7/2019

- removed WB membrane from antibody solution
- washed 6 x 5 min with 1 X TBS
- incubated membrane with sec. AB goat-anti-mouse 1:5,000 in 5 % Milk at RT for 2 h
- washed 4 x 5 min with 1x TBS
- used 800 µl staining solution (400 µl sol. A and 400 µ sol. B)
- no signal: eventually overheating
- Preparation of WB under non-reducing conditions
- Laemmli: 0.125 M Tris/HCl + 4 % SDS + 15 % Glycerol + 2 % Phenol Red
- diluted samples 1:2 with Laemmli (vol 20µl)

WesternBlot

	A	B	C	D	E	F	G	H
1	E1a FT 1:50	E1a W1:5	E1a E 1:1	E1a E1:50	E3a FT 1:50	E3a W1:5	E3a E1:1	E3a E1:5

incubated 10 min 95 °C
incubated over night at 4 °C

Repeat of VLP cell samples DNase treatment with modified protocol
To the 20 µl RNA extraction sample of the VLPs from -80 °C fridge (purification started friday 12.07.19) are given
610 µl 10 x reaction buffer
62 µl DNase 1
the incubation time at 37 ° C was extended to 1 h. Then 2x Phenol-Chloroform-extraction + RNA precipitation in EtOH over night at -20 °C
Because the lysation of the supernatant samples (VLPs) from 12.07.19 seems to have failed, the lysed supernatant the same samples was taken and the RNA extraction procedure was repeated
DNase treatment
150 µl sample
15 µl 10 x Reaction buffer
3 µl DNase 1
Incubated for 1 h at 37 °C
Phenol-Chloroform-treatment: due to high protein content Phenol-Chloroform addition was performed 3 times then Chloroform and EtOH precipitation over night