# 09. (September) 2019

**Project:** iGEM_Munich2019 Shared Project
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- continued with WB
- washed 3x10min in TBST
- 2h at RT in sec AB diluted - goat-anti-mouse 1:20000 in 5% BSA
  goat-anti-rat 1:10000 in 5% BSA
  dilution to try to reduce background

  new WB VLP-anti-MCP
  - harvested samples like VLP harvest protcol
  - lysed all samples dilution 1:1
- dilutede 1:1 with laemmli, incubated 10 min at 95 °C
- SDS-Page and WB after protocol

**Table3**

| | A | B | C | D | E | F | G | H | I | J | K | L | M | N | O |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | marker | Mock SNL | mock CL | V4 SNL | V4 CL | MCP-MS2 3 SNL | MCP-MS2 3 CL | 3* SNL | 3* CL | 4 SNL | 4 CL | 5 SNL | 5 CL | 6 SNL | 6 CL |

VLP samples received
750 µl supernatant and all cells are used for lysis and DNase treatment

RNA extraction (Exosomes, VLPs and correspondin cells)

the standard trizol protocol

- 750 µl SN were mixed with 750 µl Trizol
- after addition of chloroform, the eppi was shaken heavily for 3 min and the incubated at RT for 5 min
- after centrifugation only 700 µl aquaous phase were transfered to new eppi
- 800 µl Isopropanol were added
- 30 min incubation at - 20 °C
- all 20 µl SN were discarded and the pellet + 20 µl SN were incubated at 60 °C for 20 min
- further 20 µl RNA se-free water were given to the samples and incubated again at 60 °C for 10 min
- the samples were frozen at - 80 °C via liquid N2 freeing