07. (July) 2019

Project: iGEM_Munich2019 Shared Project
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TUESDAY, 30/7/2019

exosome precipitation

- take exosomes out of the fridge and alliquot three 2 ml eppis
- centrifuge for 1 h at 4 °C at 10.000 x g
- discard supernatant and resuspend the non-visble pellet in PBS
- fluc-assay of orgin cells from exosomes
- tage cells out of well by pipetting medium up and down
- transfer 50 µl of cell resuspension into black 96 well plate
- add 50 µl of one glo reagent
- shake and incubate for 5 min and measure at plate reader

- RNA isolation of cell from exosomes out of 6-well-plate
- done on monday 29.07
- RNA isolation with TRIzol was don on monday but without a specific lyse or DNAse treatment prior to TRIzol RNA isolation -> TRIzol was directely added to the cell layer in the 6 well plate

- on tuesday 10 µl of DNase 1 and 10 µl of DNase1 buffer to 80 µl ddH2O was addad to the 30 µl of isolated RNA + DNA from monday incubated at 37 °C for 80 min
- After DNseI treatment 200 µl TRIzol is added and stored in -20 °C over night

- RNA isolation of exosomes supernatant of cell out of 6 well plate precipitated
- lyse of exosomes: addition of 1 % Triton lysis buffer in relation 1:1 with sample incubate for 10 min at 60 °C
- DNase treatment: addition of 10 µl of DNase 1 and 30 µl fo DNase I buffer to 400 µl sample incubated at 37 °C for 80 min
- Trizol RNA isolation addition of 400 µl TRIzol stored in -20 °C over night go on with protocol on wednesday 31.07

- WB protocol SDS page and WB

gel/blot scheme

	A	B	C	D	E	F	G	H	I
1	marker	SN neg. ctr	L 1:1	L 1:10	L 1:100	FT 1:1	FT 1:10	W1:1	E 1:1

Ponceaus staining 5 min destaining 1 min with water, complete destaining with TBS for 30 min
Blocking 1 h with 5 % Milk in TBS
Incubation in primary ab over night at 4 °C

continued WB protocol wiht membrane form 29.07
washed 3x10 min in TBS
incubated in sec AB goat anti mouse HRP for 2 h at room temperature
washed 4x5 min in 1x TBS
deveopment according to protocol
no bands could be seen

to check wether AB is responsible blot is stripped and His-Tag is targeted

- wash 3x5 min with TBS
- washed 15 min with stripping buffer
- washed 3x 5 min with TBS
- blocked in 5 % milk in TBS
- incubated in nti-n-His overnight at 4 °C

RNA isolation from exosomes supernatant samples

- exosomes purified with kit
- resuspended in 200 µl PBS
- 200 µl 1% triton added
- 10 min at 60 °C
- DNase treatment (^x buffer, 10 µl DNaseI)
- 1 h at 37°C
- stored overnight at -20 °C