

10. (October) 2019

Project: iGEM_Munich2019 Shared Project

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THURSDAY, 10/10/2019

Anja

Glycerol stock preparation: E10, E19, E20, V15 (see 04.10.2019)

Final glycerol stock for EXOs:

- E10, E19, E20, E11, E16, E17, E18, V15, (+ V14, E6, E12 present previously)

Cytotoxicity (LDH) Assay 24h after transfection (according to Cytotoxicity (LDH) Assay protocol)

-3 technical replicates were taken from each well

-Triton-X-100-PI-DMEM mix volume adjusted to volume in 24-well plates (scale-up)

-done with background controls including the only full DMEM mixes

Joshi

MIN6-K8 harvesting from 24-well plate (same as HEK293T on 09.10.2019)

-cells were washed with PBS

Titration curve experiment harvesting

-120µl harvested and centrifuged (according to VLPs and exosome harvesting)

-50µl of Total Exosome Isolation reagents added to 100µl supernatant (top part after centrifugation)

-incubation in the fridge ON

HEK 293T harvesting for long-term cell-monitoring experiment from 24-well plate for qPCR analysis (24h after transfection)

-600µl harvested and centrifuged (according to VLPs and exosome harvesting)

-175µl of Total Exosome Isolation reagents added to 350µl supernatant (top part after centrifugation)

-incubation in the fridge ON

EXO HiBiT Assay for HEK293T cell line long-term cell-monitoring experiment from 24-well plates (24h after transfection, according to VLPs / Exosomes harvesting & HiBiT assay from 24-well plates)

-40µl used for supernatant lysed (SNlysed)

-10µl used for supernatant unlysed (SNunlysed)

Cell culture: Cytotoxicity assay medium change on long-term cell-monitoring plate, 48 and 72h plates (samples including or not including Zeocin™ antibiotic in the media)

-long-term cell-monitoring plate: 600µl harvested and saved for HiBiT and qPCR analysis, 600µl fresh media with/out antibiotic added

-cytotoxicity assay 48 & 72h plates: 600µl removed, 600µl fresh media with/out antibiotic added

10102019_Zeocin_treatment_mix		
	A	B
1	Component	Volume [µl]
2	Zeocin™ Stock (100mg/ml to reach 300µg/ml final conc.)	2,55
3	full DMEM	597,45

10102019_Zeocin_treatment_long_term_plate						
	1	2	3	4	5	6
A	DPBS	DPBS	DPBS	DPBS	DPBS	DPBS
B	DPBS	Zeocin + full DMEM	Zeocin + full DMEM	Zeocin + full DMEM	DPBS	DPBS
C	DPBS	full DMEM	full DMEM	full DMEM	DPBS	DPBS
D	DPBS	DPBS	DPBS	DPBS	DPBS	DPBS



10102019_Zeocin_treatment_ctx_48_and_72h_pla...						
	1	2	3	4	5	6
A	DPBS	DPBS	DPBS	DPBS	DPBS	DPBS
B	DPBS	Zeocin + full DMEM	Zeocin + full DMEM	Zeocin + full DMEM	only Zeocin + full DMEM	DPBS
C	DPBS	full DMEM	full DMEM	full DMEM	only full DMEM	DPBS
D	DPBS	DPBS	DPBS	DPBS	DPBS	DPBS



VLP HiBiT Assay for HEK293T (see entry in VLP lab book)

-overtaken before heat lysis step, data saved as VLPcells, VLPSNlysed, VLPSNunlysed (according to VLPs / Exosomes harvesting & HiBiT assay from 96-well plates)