10. (October) 2019

Project: iGEM_Munich2019 Shared Project
Authors: Anja

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Anja

Cytotoxicity (LDH) Assay 48h 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 and Zeocin+full DMEM mix wells

Joshi

HEK 293T harvesting for long-term cell-monitoring experiment from 24-well plate for qPCR analysis (48h 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 long-term cell-monitoring experiment from 24-well plate (48h 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 72h plate (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
Cell culture: Splitting and seeding
-splitted 1:6
-seeded 1x 24-well plate with 150.000 cells/well into 800µl full DMEM