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Sequential Transfection and Transduction Protocol For HUVECs (Lipo2000 version) + HALO tag live microscopy V.1

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protocol .

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4 Day Protocol for transfecting lipofected plasmids, followed by viral transduction and pre live microscopy treatment. Optimized for blood flow simulation experiments using 0.4 Ibidi uslides with HUVECs.

Emir Bora Akmeriç 2022. Sequential Transfection and Transduction Protocol For HUVECs (Lipo2000 version) + HALO tag live microscopy. **protocols.io** https://protocols.io/view/sequential-transfection-and-transduction-protocol-b3i5qkg6

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Pre Transfection

1 Seed 500k cells to a t25 flask a day(to 1.5 days max) prior, the plate on day of transfection should be 50-70% confluent 1d

2 2 hours prior to transfection, change media with EGM2 **WITHOUT antibiotics!** 2h

Transfection 2h 30m

3 Prepare the following mixes:

Mix1: 325 uL opti-mem+ 5 uL Lipo2000
Mix2: 325 uL opti-mem + 8 ug Kank1:GFP Plasmid

4 Incubate for 5 mins at RT 5m

5 Mix the tubes, incubate for 20 mins at **37C** 20m

6 Add the mix to the semi confluent t25, incubate for 2hours inside the incubator 2h 5m

7 Exchange the media with 4 mL EGM2(probably without antibiotics), incubate overnight 1d

Transduction

8 Warm EGM2 media with antibiotics 10m

9 Check cells, ideally with an epifluorescence microscope to see if GFP transfection worked

10 Add 2.66 mL EGM2 with antibiotics 10m

11 Bring the flask to S2 in a styrofoam box, suit up

12 Thaw a F1 VEC-Halo AAV, note which tube you are thawing (ideally only thaw a tube 5-6 times)

13 Add 53 uL of the virus

14 Incubate overnight 1d

Seeding 2h 20m

15 Warm up 2% gelatin in water bath. 5m

16 Dilute 1:10 to a total volume of 300 uL 5m

17 Add 120 uL of the diluted mix to 2 IBIDI ibitreat luer 0.4 flow slides(uslides), incubate 1-2 hours at 37C 2h

18 Bring a 50 mL falcon of EGM2 without antibiotics to S2, prewarmed. 5m

19 Wash cells 3x with 4mL of EGM2 and add 4mL media again 5m

20 Harvest transduced/transfected cells and bring to a concentration of 2.25 million/mL

21 Add 100 uL of cell suspension, work fast and avoid bubbles

22 Incubate for 30 minutes

23 Add 120 uL of EGM2+antibiotic to the reservoirs, incubate overnight

Pre Live Microscopy Treatment 45m

24 Prewarm CO2 independent media with GA antibiotic and thaw a 200 uM Janelia Fluor 549 HaloTAG

25 Dilute halotag 1:200 with warm media, incubate for 5 mins. Then dilute 1:5 again.

26 Add 220 uL of media per slide. Incubate for 45 mins 45m

27 Meanwhile, put a styrofoam box in the incubator for moving.

28 Gently remove media and carefully add an equal volume. Transfer slides to the imaging facility