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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.

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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<sup>2h</sup> at 37C
- 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