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Transfection of primary endothelial cells with siRNA

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Protocol status: Working

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Preparing the endothelial cells for transfection (12-well pla..

- 1 Cells were seeded the day before transfection at a density of 3×10^4 cells/cm².

Transfection

- 2 Cells were washed twice with PBS and then starved for at least 02:00:00 in 500 µL serum-free basal MCDB131 Medium at 37 °C 00:20:00 before incubation is finished prepare the transfection mixture (volumes are for a single well of a 12-well plate)
 - in one tube (A) add 1.5 µL of siRNA (50 pmol stock) to 125 µL Opti-MEM
 - in a second tube (B) add 3.75 µL of Lipofectamin RNAiMax into 125 µL Opti-MEMMix each tube gently and incubate at room temperature for 00:10:00
After incubation the siRNA solution from tube A was added to the lipofectamin solution in tube B and incubated for 00:10:00 at room temperature
225 µL of the siRNA/lipofectamin solution was added to each well
After 04:00:00 to 06:00:00 the medium was changed to endothelial cell growth medium
The endothelial cells were incubated for 48:00:00 at 37 °C then washed with PBS and snap frozen in liquid nitrogen for further analysis