

Transfection of miRNA mimics in HUVECs

Reagents

cell types:	HUVECs
miRNA inhibitor:	miRVana miRNA mimics 5 nmol, dilute 5 nmol in 250 µl H ₂ O = 20 µM stock solution, aliquot, store at -20°C
transfection reagent:	Lipofectamine RNAiMAX (Invitrogen, Cat.no. 13778-075), 4°C
transfection medium:	serum-reduced Opti-MEM I medium (Invitrogen Cat.no. 31985-062)
culture medium:	HUVEC medium (Promocell Cat.no. C-22110 + +3% FCS + 1 % Pen/Strep)
protocol:	traditional protocol
material:	vortexer, round-bottom tubes (BD Falcon, Cat.no. 352063)

Procedure

Day before transfection

6-well

- seed approx. 5×10^5 cells/in 3 ml complete medium on gelatine-coated wells

Day of transfection

Mix1

- prepare as master mix for each well to be transfected: 250 µl OptiMEM + 5 µl RNAiMAX per well in round-bottom tubes: pre-load with OptiMEM
mix Lipofectamine gently prior to addition to OptiMEM

Mix 2

- combine Opti-MEM with RNA tool (miRNA mimic, miRNA inhibitor) in Eppi

for 1x 6-well

Mimic	Untrans	1 nM Mimic
Mix 1 OptiMEM RNAiMAX	250 µl 5 µl	
Mix 2 OptiMEM miR Tool	250 µl	247 µl 3 µl of 1 µM
Complex added to ml Opti-MEM	2.5 ml	2.5 ml

- combine Opti-MEM/RNAiMAX with Opti-MEM/RNA tool in round-bottom tube (Mix 1 +2)
mix gently and incubate for 15 min at RT
- remove culture medium from HUVEC, wash once with PBS, add 2.5 ml Opti-MEM (without FCS or supplements)
per 6-well
- drop-wise add Lipofectamine/miRTool mixture to HUVECs, mix by rocking the plate back and forth, incubate at 37°C
- 4h post-transfection: change Opti-MEM-based transfection medium to complete HUVEC medium

1st Day after transfection

- change medium 24 h post-transfection, if required