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Working

Transfection of primary endothelial cells with siRNA

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THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

Epigenetic control of the angiotensin-converting enzyme in endothelial cells during inflammation PLOS ONE

Preparing the endothelial cells for transfection (12-well plate)

1 Cells were seeded the day before transfection at a density of 3 x 10^4 cells/cm^2.

Transfection

2 Cells were washed twice with PBS and than starved for at least **02:00:00** in **500 µl** serum-free basal MCDB131 Medium at **37 °C**

00:20:00 before incubation is finshed 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-MEM

Mix each tube gently and incubate at room temperatur 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** the washed with PBS and snap frozen in liquid nitrogen for further analysis