RNAi on SmGPCR transfected HEK (siPORT Amine)

(1) 24hrs prior to transfection with siRNA, plate (0.2-1.0) x 10^5 cells/well of 24well/plate in DMEM+ 10% FBS without selective antibiotics that used to be used SmGPCR expression. **I plate 100,000cell/well.**

(2) Next day, vortex siPORT Amine tube. Mix **47µl OPTI MEM + 2µl siPORT Amine** (for each well). It is better to prepare a master mix to minimize pipetting error. **Incubate the diluted siPORT Amine at RT for 20min.**

(3) Add **0.13-1.25µl** of in vitro synthesized **siRNA** to OPTI MEM/Amine to get final conc **1-25nM siRNA/well**. Incubate for 20min at RT.

(4) Remove old DMEM/FBS and **add new 200µl DMEM+ 10% FBS/well**

(5) Add Amine/siRNA complex dropwise onto cells. Gently rock the plate back and forth to evenly distribute the medium.

(6) Incubate cells in this transfection medium for 4-24hrs.

(7) Add fresh DMEM+ FBS after that transfection period in last step (Do not remove the transfection medium). Incubate for 3-6days.

Calculations:

My stock siRNA SmGPCR is 35µM (i.e. 35,000nM) and the commercial scrambled GAPDH 50µl (50,000nM).

The following is sufficient to prepare transfection medium for two wells (1well for each SmGPCR and GAPDH siRNAs). For duplicates or more, prepare more medium.

In step 2: 98µl total: 94µl of OPTI MEM + 4µl siPORT Amine and incubate at RT/20min

In Step 3: Divide the 98µl into two halves (each with 49µl) and add 0.1µl of stock siRNA GAPDH in one tube and 0.143µl of siRNA SmGPCR in the second half. Incubate at RT for 20min.

I will add 500µl of fresh DMEM+ 10% FBS to each well after the 4hr transfection incubation. This will make the volume of 750µl and the concentration of **6.7nM**. This will be added as 250µl today (after 4hrs of transfection) and after 2days, another 250µl.

RNAi on SmGPCR transfected HEK (siPORT Lipid)

(1) 24hrs prior to transfection with siRNA, plate (0.2-1.0) x 10^5 cells/well of 24well/plate in DMEM+ 10% FBS without selective antibiotics that used to be used SmGPCR expression. **I plate 100,000cell/well.**

(2) Next day, vortex siPORT Lipid tube. mix **5.5µl OPTI MEM + 2µl siPORT Amine** (for each well). It is better to prepare a master mix to minimize pipetting error. **Incubate the diluted siPORT lipid at RT for 20min.**

(3) Add **0.13-1.25µl** of in vitro synthesized **siRNA** to OPTI MEM to get final volume **42 µl**. Incubate for 20min at RT.

(4) Add diluted siRNA to diluted siPORT lipid (i.e **7.5 µl + 42 µl → 49.5 µl). Incubate at RT for 20min.**

(5) Remove old DMEM/FBS and **add new 200µl OPTI MEM/well**

(6) Add Lipid/siRNA complex dropwise onto cells. Gently rock the plate back and forth to evenly distribute the medium.

(7) Incubate cells in this transfection medium for 4hours.

(8) Add 0.25ml fresh DMEM+ FBS after that transfection period in last step (Do not remove the transfection medium). Incubate for 3-6days.

Calculations:

My stock siRNA SmGPCR is 35µM (i.e. 35,000nM) and the commercial GAPDH 50µl (50,000nM). Incubate at RT for 20min.

The following is sufficient to prepare transfection medium for two wells (1well for each SmGPCR and GAPDH siRNAs). For duplicates or more, prepare more medium.

In step 2 (diluted siPORT lipid): 15µl total as 11µl OPTI MEM + 4µl siPORT Lipid.

In Step 3 (diluted siRNA): 42µl total as 0.1µl GAPDH stock + 41.9µl OPTI MEM or 0.143µl SmGPCR + 41.857µl OPTI MEM. Incubate at RT for 20min.

Step 4: Add 7.5µl of step 2 (i.e. half) to each tube made in step 3→ 49.5µl total and incubate for 20min/RT.

I will add 500µl of fresh DMEM+ 10% FBS to each well after the 4hr transfection incubation. This will make the volume of 750µl and the concentration of **6.7nM**. This will be added as 250µl today (after 4hrs of transfection) and after 2days, another 250µl.