siRNA transfection protocol with HiPerFect (Qiagen 301705)

General considerations:

Dissolve siRNA in sterile water and prepare 5 µl aliquots, store at –80°C.

For use, dilute siRNA in siRNA dilution buffer (diluted siRNA is not stable, use only once). 5x siRNA buffer aliquots (Dharmacon) are stored at 4°C in the cell culture lab. Dilute 5x siRNA buffer to 1x siRNA buffer before use using RNase-free sterile Sigma H_20.

Use RNAse-free tips, tubes, water etc. (freshly autoclaved).

For 1st experiment, you must determine knockdown efficiency by semiquantitative RT-PCR (typically, 18, 26, 34 cycles, starting from 1-2 µg of total RNA for cDNA synthesis). RNA analysis is being carried out 24 and 48h after transfection.

Routine procedure is 1 round of RNAi, however, if proteins have long half-life times, a second round may be necessary. In this case, repeat transfection 24-36h after first transfection.

Amplicons for GAPDH must be used for standard.

For positive transfection control, use YFP DNA in a separate transfection.

For positive control of knockdown efficiency, use siRNA against X.

As negative control, use siRNA against GFP (if the cells are not stably transfected with GFP).

Experimental setup (for 3 cm dishes):

Number of cells per dish: a. seeding day before transfection 0,8-3 x 10^5

b. seeding day of tranfection 1,5- 6 x 10^5

Cells should be in optimal physiological condition on the day of transfection (no trypsinization damage etc., otherwise stop experiment). Optimal confluency for transfection of adherent cells with this protocol is 50-80%. If the analysis (eg immunofluorescence) requires much lower cell density, trypsinize cells 1-2 days after transfection.

Follow standard protocol (use 3cm dish or 6 well plate for pilot experiment, see pipetting scheme for optimizing transfection in handbook):

1. Change medium, add 2.3ml fresh medium per dish.

2. Dilute 12.0 µl 10µM siRNA (= 50nM final) in 100 µl OptiMEM.

3. Add 12 µl HiPerFect to diluted siRNA and mix by vortexing 3 x 1 sec., vortex pos. 1.

4. Incubate at room temperature for 5-10 minutes.

5. Add mixture dropwise onto the cells. Gently swirl the plate.

6. Incubate at 37° C (it is not necessary to change the medium after transfection).

7. Media change 24 h post-transfection.

<u>Analysis</u>:

Gene silencing starts 6h after transfection, but: proteins can be very stable and therefore, effects at protein level may be visible only after 60-90h.

RNA: 24 and 36 h after transfection.

Immunofluorescence: 24, 48, 72, 96h (you may have to trypsinize cells, depending on growth rate).

Western blot: 24, 48, 72, 96h. This can be done from the same dish that holds coverslips for IF.

For upscaling and optimization, check handbook.

For general considerations of RNAi, refer to Current Protocols.