

Transfection of miRNA into mammalian monolayered cells

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Seed $4-5 \times 10^5$ cells in a well of 24-well plate, which constitutes approximately 40 – 60 % confluency, and incubate for 18 hr.

Make the following mixture and mix gently.

miRNA of interest (15 nM)	1 μ l
FITC-labeled siRNA (15 nM; Bioneer # SN-1021)	0.1 - 0.2 μ l
Opti-MEM (or your preferred media without serum)	50 μ l

Make the following mixture, mix gently and incubate for 5 min at RT[#].

Lipofectamine 2000* (Invitrogen # 11668-027)	1 μ l
Opti-MEM (or your preferred media without serum)	50 μ l

Combine the diluted miRNA with the diluted Lipofectamine 2000. Mix gently and incubate for 20 min at RT.

Add the complex to each well and swirl the plate for mixing.

Four hr[%] after the transfection, observe the cells under fluorescent microscope to estimate transfection efficiency.

Incubate the cells for at least 72 hr and then harvest them. If media turns orange during the 72-hr incubation, replace media with fresh one.

(Optional) If conditioned media should be harvested for zymography, change media at 48 hr after the transfection, incubate cells further for additional 24 hr and then harvest the conditioned media.

* Vortex briefly before use.

For further info, refer to

http://tools.invitrogen.com/content/sfs/manuals/lipofectamine2000_man.pdf

% After 4 hr, fluorescent signal may diminish.