miRNA mimic transfection with Lipofectamine RNAiMAX

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Abstract

This protocol is provided for optimization of miRNA mimics transfection in a 6-well plate. Lipofectamine® RNAiMAX Transfection Reagent is a proprietary formulation for transfecting small RNAs (e.g., siRNA, Silencer® Select siRNA, Stealth® RNAi, mirVana™ miRNA mimics and inhibitors) into a wide range of eukaryotic cells.

Citation: Alessandra Bisagni,Stefania Croci miRNA mimic transfection with Lipofectamine RNAiMAX. **protocols.io** dx.doi.org/10.17504/protocols.io.n58dg9w
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Guidelines

RNA-Lipofectamine® RNAiMAX complexes must be prepared in serum-free medium such as Opti-MEM® Reduced Serum Medium and can be added directly to cells in culture medium, in the presence or absence of serum and antibiotics. It is not necessary to remove complexes or change/add medium after transfection unless toxicity by Lipofectamine® RNAiMAX is observed.

Protocol

Step 1.

Seed 2x10^5 cells into a 6-wells plate with 2 mL RPMI + 10% FBS plus antibiotics (Day 0).

Step 2.

Plated cells has to be 60-80% confluent at the time of transfection. (Day 1).

Step 3.

Dilute 5.0 µL Lipofectamine® RNAiMAX Reagent in 125.0 µL Opti-MEM® Medium per each well that has to be treated.

Step 4.

Dilute 2.0 µL miRNA in 125.0 mL Opti-MEM® Medium per each well that has to be treated.

Step 5.

Add 125 µL diluted miRNA to 125 µL diluted Lipofectamine® RNAiMAX Reagent (1:1 ratio).

Step 6.

Incubate both solutions for 5 minutes at room temperature.

Step 7.

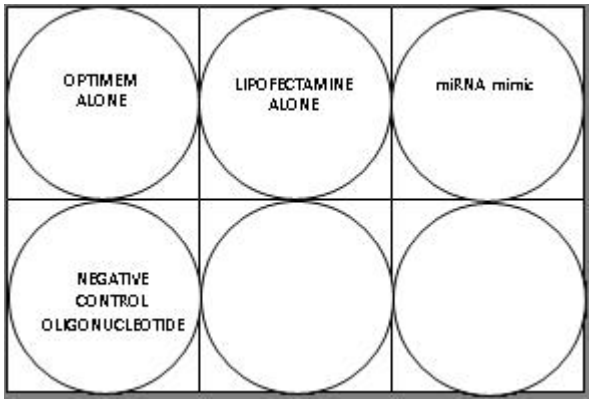
Change the medium from cells to remove dead cells.

Step 8.

Add 1750 µL fresh medium.

Step 9.

Add 250 µL of relative miRNA Lipofectamine complexes to the wells as shown in figure belove.



Step 10.

Incubate cells for 1–3 days at 37°C. Then, analyse transfected cells.

Note: As controls it is important to include cells treated with Optimem alone, cells treated with Lipofectamine alone (to evaluate the toxicity of the transfetion reagent) and cells treated with a negative control oligonucleotide to evaluate aspecific effects.