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We use this protocol and it's working

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siRNA Transfection of Dispersed Islet Cells

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ABSTRACT

siRNA Transfection of Dispersed Islet Cells

MATERIALS

Silencer™ FAM-labeled Negative Control No. 1 siRNA
Catalog number: AM4620 (ThermoFisher)

Opti-MEM™ I Reduced Serum Medium
Catalog number: 31985070 (Gibco)

Lipofectamine™ RNAiMAX Transfection Reagent
Catalog number: 13778075 (ThermoFisher)

Accell Non-targeting Control Pool
Catalog ID: D-001910-10-05

siRNA of your target gene

BEFORE START INSTRUCTIONS

Reconstitute your siRNA to a 20 µM working solution with nuclease-free water.

Work in a cell culture hood, in an RNAse-free environment, and keep the hood light off.

Clean the cell culture hood, turn the UV light on, and thaw reagents on ice.

1 Disperse islets, and resuspend cells in Opti-Mem media after centrifugation. Distribute 100 µL of media with cells per 35 mm dish.

Remember to make extra dishes for qPCR to test the efficiency of the transfection.

2 For each control dish (calculate the volume of reagents according to the number of dishes you wish to make):
Tube 1: 50 µL OptiMem media + 3 µL Lipofectamine RNAiMAX Transfection Reagent
Tube 2: 50 µL OptiMem media + 0.6 µL control siRNA + 0.4 µL dye indicator (Silencer™ FAM-labeled Negative Control)

For each dish with the target gene silenced:
Tube 3: 50 µL OptiMem media + 3 µL Lipofectamine RNAiMAX Transfection Reagent
Tube 4: 50 µL OptiMem media + 0.6 µL target siRNA + 0.4 µL dye indicator (Silencer™ FAM-labeled Negative Control)

Wait 5 minutes after making the 4 tubes.

3 Mix tubes 1 and 2, and tubes 3 and 4. Wait 15 minutes.

4 Add 103 µL of transfection reagents to each dish with 100 µL of media with dispersed cells.

5 Wait 4-6h, and add 2 ml of human media (DMEM) to the dishes.

6 After 2-3 days, perform desired experiment and add 0.5 -1 mL of Trizol to the extra dishes. Freeze cells with Trizol in the -80°C freezer until RNA extraction.