PROTOCOL

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INSPIRED CELL SOLUTIONS

Reverse Transfection (RTF) Optimization Plates

This protocol is for the Dharmacon RTF Optimization Plates.

Introduction

Optimizing RNA interference (RNAi) experiments is a critical component to successful gene silencing experimentation. The Reverse Transfection Format (RTF) Optimization plates are designed for the optimization of siRNA experiments prior to using Dharmacon™ siGENOME™ or Dharmacon™ ON-TARGET*plus*™ RTF siRNA Libraries.

Product description

Once the optimum Dharmacon™ DharmaFECT™ siRNA transfection reagent (or other transfection reagent of choice) has been determined for your cell line, the RTF Optimization Plates allow simple and flexible determination of (a) the best negative control siRNA, (b) optimal cell density and (c) DharmaFECT transfection reagent amount for the cells and assay being used. The pre-plated controls provided at 6.25 pmol per well in triplicate plate sets are described in the accompanying Product Insert.

Each RTF Optimization Plate is provided with pre-plated controls as described below (Figure 1).

- Rows A-D contain four different Non-targeting siRNAs.
- Rows E contains a Non-targeting siRNA pool.
- Row F contains a validated positive control siRNA pool targeting the housekeeping gene Cyclophilin B (PPIB).
  a. Row G is recommended for mock transfected (or transfection reagent alone) to determine if there is any effect of the transfection reagent on cell viability and/or mRNA expression.
  b. Row H is recommended for untreated cells and serves as 100% viability control and/or 100% mRNA level control.

Handling precautions

Oligonucleotides are susceptible to enzymatic degradation by nucleases and to chemical degradation by extreme pH and temperatures. Wearing gloves and maintaining nuclease-free conditions when handling the oligonucleotides is highly recommended.

Additional materials required for use

- DharmaFECT Transfection Reagent 1, 2, 3 or 4 (Cat #T-2001, T-2002, T-2003, T-2004)
- Serum-free and antibiotic-free cell culture medium such as MEM-RS, Hyclone Cat# SH30564
- Equipment, reagents and supplies needed for cell culture
- Equipment, reagents and supplies needed for transfection efficiency assessment
- Deep-well plate for preparation of the Master Dilution Plate [for example Nunc™ 96 DeepWell Plate, 1.0 mL, Polystyrene, Sterile, Transparent, from Fisher Scientific (Cat #12-565-553)]

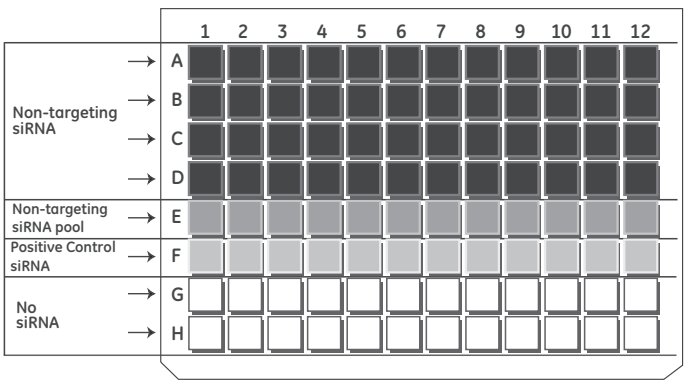

Figure 1. RTF Optimization Plate Layout.

Protocol

The recommended experimental format for RTF Optimization Plates is shown in Figure 2. The Dharmacon DharmaFECT transfection reagent that has been predetermined to be optimum for the cells (from use in conventional transfection experiments) is tested at two volumes with two cell numbers per well. An alternate method could assess four DharmaFECT reagent volumes with a single cell number per well.

The instructions below describe how to construct the plate format shown in Figure 2. All manipulations should be performed under sterile, RNase-free conditions. The final siRNA concentration during transfection is 50 nM when using this protocol.

1. Allow RTF Optimization Plates and serum-free and antibiotic-free cell culture medium to equilibrate to room temperature.
2. Prepare the Rehydration Solution in two 15 mL tubes as described below and in Figure 2. Tube X will prepare reagent for a final volume of 0.15 µL DharmaFECT reagent per well and Tube Y will prepare reagent for a final volume of 0.075 µL DharmaFECT per well.
   a. Dispense 3519 µL serum-free and antibiotic-free cell culture medium into Tube X and 1180 µL into Tube Y.
   b. Add 21 µL DharmaFECT Transfection Reagent to Tube X and mix well by pipetting up and down.
   c. Transfer 1180 µL from Tube X to Tube Y. Mix well.

Tubes	Volume of (µL)	Volume of DharmaFECT (µL)
X	3519	21
Y	1180	None

   d. Prepare Master Dilution Plate by dispensing 330 µL from each Rehydration Solution tube to the appropriate wells (shown below) in a deep-well plate, and 330 µL of serum-free and antibiotic-free cell culture medium into wells H1 and H2. Following the recommended protocol will facilitate convenient and accurate dispensing of the Rehydration Solution to the RTF Optimization Plate. The Rehydration Solution may be stored under sterile conditions at room temperature for up to 2 hours prior to use.
3. Rehydrate wells of RTF Optimization Plate by transferring 25 µL of cell culture medium or Rehydration Solution from the appropriate wells of the Master Dilution Plate as described below and in Figure 2.

Tubes	Dispense into Wells of Master Dilution Plate
X	A1 – G1
Y	A2 – G2
Cell culture medium	H1 and H2

Note: The Rehydration Solution may not coat the bottom of the wells but rather "ring" the bottom of the well. This is expected and does not interfere with rehydration of the siRNA.

4. After the transfer of Rehydration Solution to the RTF plate is completed, allow the DharmaFECT transfection reagent to complex with siRNA by incubating the rehydrated RTF Optimization plates at room temperature for 15 to 90 minutes. Optimum incubation time is 30 minutes.
5. Add cells to rehydrated RTF Optimization Plates:
   a. Prepare at least 5.5 mL of cell suspension in antibiotic-free complete medium. The cell concentration should be such that when 100 µL is dispensed into each well, the required cell number per well is achieved. For example, to add 1×10^3 cells per well, the cell suspension should have 1×10^4 cells per mL, and to add 2×10^3 cells per well, the cell suspension should have 2×10^4 cells per mL.
   b. Add 100 µL of the low cell number cell suspension to Columns 1–6 and add 100 uL of the high cell number cell suspension to columns 7–12. The final volume in each well will be 125 µL. The final concentration of siRNA is 50 nM.
6. Incubate the RTF Optimization Plates under appropriate culture conditions (for example 37 °C, 5 % CO_2).
   - For the most consistent results, we recommend that RTF plates be incubated for at least 2 days before performing the transfection efficiency and screening assays of choice.

From Master Dilution Plate	To Optimization Plate
Wells A1 – H1	Wells A1 – H1
	Wells A2 – H2
	Wells A3 – H3
	Wells A7 – H7
	Wells A8 – H8
	Wells A9 – H9
Wells A2 – H2	Wells A4 – H4
	Wells A5 – H5
	Wells A6 – H6
	Wells A10 – H10
	Wells A11 – H11
	Wells A12 – H12

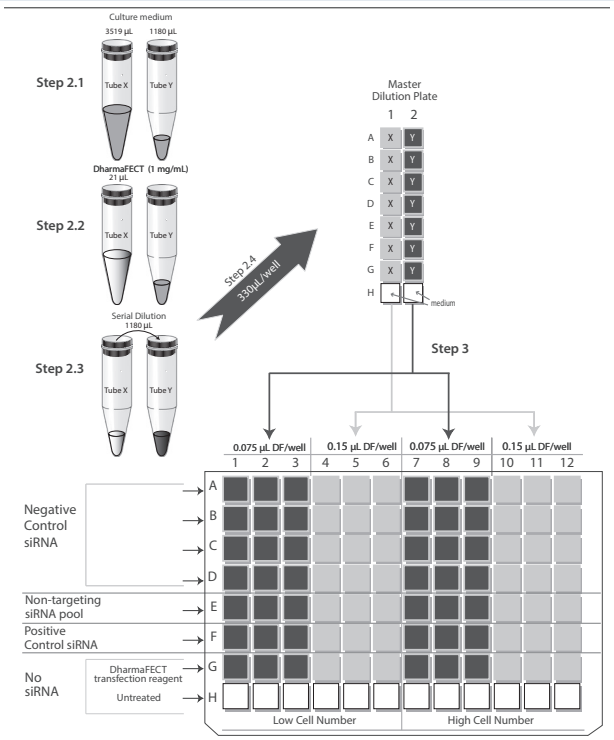

Figure 2. RTF Optimization Plate Rehydration Guide.

If you have any questions, contact

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