










48-Well Plate siRNA Knockdown Optimization Protocol for Suspension Cells

Day	Step	Visualized Step	Instruction	Component	Details											
Day: 0	Step 1: Culture	100k - 150k cells/mL 	Culture cells in flask	Suspension Cells, media	Resuspend cells to a concentration of 100,000 – 150,000 cell/mL											
Day: 1	Step 2: Dilute siRNA	12 x 	Dilute stock siRNA in serum-free media in 12 tubes by pipetting up and down	12 x Tubes	siRNA Groups											
				siRNA Volume (µL)	1	2	3	4	5	6	7	8	9	10	11	12
				Media Volume (µL)	229-*	226-*	324-*	321	313	305	314.5	302.4	290.4	308	292	275.9
	Step 3: Add Reagent	12 x 	Vortex stock Transfection Reagent and add directly to diluted siRNA tubes	12 x Tubes	Reagent Groups											
				Reagent Volume to add (µL)	1	2	3	4	5	6	7	8	9	10	11	12
	Step 5: Mix		Gently vortex (~3 sec) the siRNA-Reagent mixture to ensure homogenous mixing for complexation.	Experimental Group	Complex Groups											
				siRNA Concentration (µL/mL)	1	2	3	4	5	6	7	8	9	10	11	12
				siRNA : Reagent Mass Ratio	0.6	0.9	1.2	0.6	0.6	0.6	0.9	0.9	0.9	1.2	1.2	1.2
	Step 6: Complex	30 min 	Incubate siRNA-Reagent complex.	Incubate the siRNA-Reagent complexes for 30 minutes at room temperature												
	Step 7: Add	100 µL 	Add siRNA-Reagent complexes to wells	Add 100 µL of siRNA-Reagent complexes to each well (in triplicate) from each group 1 through 12												
Step 8: Add	300 µL 	Add cells to siRNA-Reagent complexes in wells	Add 300 µL of cells in suspension to each well over the 100 µL of siRNA-Reagent complexes. This should give a cell concentration of 75,000 – 112,500 cells/mL													
Day: 2 - 4	Step 9: Incubate	1-3 days 	Incubate cells/siRNA-Reagent complexes.	Incubate cells for 1 – 3 days												
	Step 10: Assay		Analyze cells	Quantify transfection to determine best combination of siRNA concentration and siRNA : Reagent ratio												

*Optional: prepare 340 µL solutions of your reference reagent sufficient to complex with 0.7, 1.05 and 1.4 µg of siRNA. X is the mass ratio of siRNA to reagent, for example if one uses 2 µg of reagent for every 1 µg of siRNA then X would be 2.

48-Well Plate siRNA Transfection Optimization Protocol for Suspension Cells

Materials Needed:

- siRNA (0.14 µg/µL ~10µM)
- Serum Free Medium (eg. DMEM, RPMI-1, αMEM)
- 12 × 1.5 mL Microcentrifuge Tubes

Purpose:

This protocol is designed to optimize transfections with RJH reagents and to select the best combination of siRNA dosage and siRNA : Reagent ratio.

It can be used to compare RJH reagents vs other transfection reagents to determine which reagents are best for your application. This can be done by preparing three 340 µL solutions of your current transfection agent that will be sufficient to complex with 0.7, 1.05, 1.4 µg of siRNA.

For Example:

A researcher typically uses a 1 µg/µL solution of lipid-based transfection reagent with 2 µg of reagent per 1 µg of siRNA to transfect cells (this is a siRNA to reagent mass ratio of 1:2). In order to complex 0.7, 1.05 and 1.4 µg of siRNA the researcher will add 1.4, 2.1, and 2.8 µL of the reagent to 5, 7.5, and 10 µL of siRNA (assuming the siRNA is at a concentration of 0.14 µg/µL) the siRNA should already be diluted in 321, 313, and 305 µL of serum-free media respectively. This will yield three separate dosages (0.5, 1, and 1.5 µg/mL of siRNA) of transfection complexes with a volume of 340 µL each. On a 48-well plate triplicate sets of wells will be treated with 100 µL of complexes and 300 µL of suspension cells in each well for a final well volume of 400 µL.

How to Use this Protocol:

After following the protocol, choose the best performing groups (1 thru 12) and use **siRNA-Reagent Group Legend** to determine the optimal reagent, siRNA dosage, and siRNA : Reagent mass ratio for your application.

For Example:

A researcher transfects cells with RJH Prime-Fect and the reference reagent from in the previous example (siRNA : Reagent mass ratio of 1 : 2). Groups 3 and 5 showed the best transfection, using the **siRNA-Reagent Group Legend**, the optimal transfection conditions are:

Group 3

Reagent = Lipid-Based Reagent
siRNA Dosage = 1.5 µg/mL
siRNA : Reagent mass ratio = 1 : 2

Group 5

Reagent = RJH Prime-Fect
siRNA Dosage = 0.5 µg/mL
siRNA : Reagent mass ratio = 1 : 8

48-Wellplate Template

	1	2	3	4	5	6	7	8
A	NT	1	3	5	7	9	11	
B	NT	1	3	5	7	9	11	
C	NT	1	3	5	7	9	11	
D		2	4	6	8	10	12	
E		2	4	6	8	10	12	
F		2	4	6	8	10	12	

siRNA-Reagent Group Legend

Group	Reagent	siRNA Dosage (µg / mL)	siRNA : Reagent Mass Ratio
1	Reference	0.5	1: $\frac{X}{4}$
2	Reference	1.0	1: $\frac{X}{2}$
3	Reference	1.5	1: X
4	RJH	0.5	1 : 4
5	RJH	0.5	1 : 8
6	RJH	0.5	1 : 12
7	RJH	1.0	1 : 4
8	RJH	1.0	1 : 8
9	RJH	1.0	1 : 12
10	RJH	1.5	1 : 4
11	RJH	1.5	1 : 8
12	RJH	1.5	1 : 12

*X is the siRNA : Reagent mass ratio of your choice