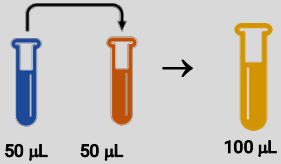
48-Well Plate pDNA Transfection Optimization Protocol for Adherent Cells

Day	Step	Visualized Step	Instruction	Component	Details											
Day: 0	Step 1: Seed	48-well 	Seed cells to be ~70% confluent for time of transfection	Adherent Cells	10,000 cells per well in 270 µL medium											
Day: 1	Step 2: Dilute Reagent	12 x 	Vortex stock Transfection Reagent and dilute in serum-free media in 12 tubes	12 x Tubes	**Reagent Groups**											
					1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
				Reagent Volume (µL)	*	*	*	0.75	1.5	3	1.5	3	6	3	6	12
				Medium Volume (µL)	*	*	*	49.25	48.5	47	48.5	47	44	47	44	38
	Step 3: Dilute DNA	12 x 	Pipette stock DNA to mix and dilute in serum-free media in another 12 tubes	12 x Tubes	**DNA Groups**											
					1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
				DNA Volume (µL)	0.6	1.2	2.4	0.6	0.6	0.6	1.2	1.2	1.2	2.4	2.4	2.4
				Medium Volume (µL)	49.4	48.8	46.6	49.4	49.4	49.4	48.8	48.8	48.8	47.6	47.6	47.6
	Step 5: Mix		Add the 50 µL of DNA to the 50 µL of reagent and mix gently	Experimental Group	**Complex Groups**											
					1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
				DNA Concentration (µL/mL)	0.3	0.6	1.2	0.3	0.3	0.3	0.6	0.6	0.6	1.2	1.2	1.2
				DNA : Reagent Mass Ratio	$1:\frac{X}{4}$	$1:\frac{X}{2}$	1:X	1:2.5	1:5	1:10	1:2.5	1:5	1:10	1:2.5	1:5	1:10
	Step 6: Complex	30 minutes 	Incubate DNA-Reagent complex.	Incubate for 30 minutes at room temperature												
	Step 7: Add	30 µL/well 	Add DNA-Reagent complexes to cells	Add 30 µL to each well (in triplicate) from each group 1 through 12 and gentle shake the plate to distribute complexes												
Day: 2 - 4	Step 8: Incubate	1 – 3 days 	Incubate cells and DNA-Reagent complexes.	Incubate cells at desired temperature for 1 – 3 days												
	Step 9: Assay		Analyze cells	Quantify transfection to determine best combination of DNA concentration and DNA : Reagent ratio												

*Optional: prepare 50 µL solutions of your current transfection reagent sufficient to complex with 0.6, 1.2, and 2.4 µg of DNA. X is the mass ratio of DNA to reagent, for example if one uses 4 µg of reagent for every µg of DNA then X would be 4.

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Materials Needed:

- Plasmid DNA (0.5 μg / μL)
- Serum Free Medium (eg. DMEM, RPMI-1, αMEM)
- 24 × 1 mL Microcentrifuge Tubes

Purpose:

This protocol is designed to optimize transfections with RJH reagents and to select the best combination of DNA dosage, and DNA : 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 30 μL solution of your current transfection agent that will be sufficient to complex with 0.6, 1.2, 2.4 μg of DNA.

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 DNA to transfect primary cells (A DNA to reagent mass ratio of 1:2). In order to complex 0.6, 1.2, and 2.4 μg of DNA the researcher will use 1.2, 2.4, and 4.8 μL of the reagent in 48.8, 47.4 and 45.2 μL of serum-free medium respectively.

How to Use this Protocol:

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

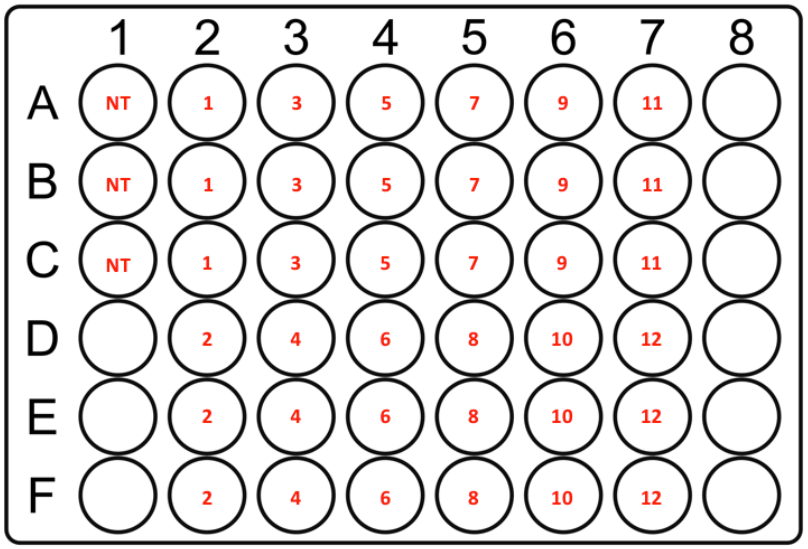
For Example:

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

Group 3
Reagent = Lipid-Based Reagent
DNA Dosage = 1.2 μg/mL
DNA : Reagent mass ratio = 1 : 2

Group 5
Reagent = RJH Prime-Fect
DNA Dosage = μg/mL medium
DNA : Reagent mass ratio = 1 : 5

48-Wellplate Template



DNA-Reagent Group Legend

Group	Reagent	DNA Dosage (μg / mL)	DNA : Reagent Mass Ratio
1	Reference	0.3	$1:\frac{X}{4}$
2	Reference	0.6	$1:\frac{X}{2}$
3	Reference	1.2	1: X
4	RJH	0.3	1 : 2.5
5	RJH	0.3	1 : 5
6	RJH	0.3	1 : 10
7	RJH	0.6	1 : 2.5
8	RJH	0.6	1 : 5
9	RJH	0.6	1 : 10
10	RJH	1.2	1 : 2.5
11	RJH	1.2	1 : 5
12	RJH	1.2	1 : 10
*X is the DNA : Reagent mass ratio			