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Lipofectamine™ LTX Optimization

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Materials Needed

- Lipofectamine™ LTX Reagent (Cat. nos. 15338-100, 15338-500)
- PLUS™ Reagent (3 µg/µL) (Cat. no. 11514-001; also supplied with Cat. no. 15338-100)
- Opti-MEM® Reduced Serum Medium (Cat. nos. 319850, 519850, or 22600)
- 20 µg of test plasmid DNA (GFP plasmid or other)
- 24-well plate containing cells of interest at optimal confluency in 500 µL of growth medium, and ready for transfection
- Round or V-bottom 96-well plate for reagent preparation (× 1)
- 1.5 mL polypropylene microcentrifuge tubes (× 5)

Protocol

1. In four microcentrifuge tubes labeled A–D, dilute DNA and PLUS™ Reagent in Opti-MEM® media to obtain the final concentrations and volumes indicated below.

   A) 398 µL of 5 ng/µL DNA and 2 µL PLUS™ Reagent
   B) 396 µL of 10 ng/µL DNA and 4 µL PLUS™ Reagent
   C) 394 µL of 15 ng/µL DNA and 6 µL PLUS™ Reagent
   D) 392 µL of 20 ng/µL DNA and 8 µL PLUS™ Reagent

   Mix well and incubate for 10 minutes at room temperature.

2. In a microcentrifuge tube labeled "LTX dilution," dilute the Lipofectamine™ LTX 1:10 in Opti-MEM® medium by adding 50 µL of Lipofectamine™ LTX to 450 µL of Opti-MEM® medium. Mix gently.

3. Following the table below, add **Opti-MEM®** media into the wells of the 96-well plate.

	1	**2**	**3**	**4**	**5**	**6**
A	46.25 µL	45 µL	43.75 µL	42.5 µL	40 µL	37.5 µL
B	42.5 µL	40 µL	37.5 µL	35 µL	30 µL	25 µL
C	38.75 µL	35 µL	31.3 µL	27.5 µL	20 µL	12.5 µL
D	35 µL	30 µL	25 µL	20 µL	10 µL	0 µL

4. Following the table below, add the diluted **Lipofectamine™ LTX** into the wells of the same 96-well plate.

	1	2	3	4	5	6
A	3.75 µL	5 µL	6.25 µL	7.5 µL	10 µL	12.5 µL
B	7.5 µL	10 µL	12.5 µL	15 µL	20 µL	25 µL
C	11.25 µL	15 µL	18.7 µL	22.5 µL	30 µL	37.5 µL
D	15 µL	20 µL	25 µL	30 µL	40 µL	50 µL

5. Add 50 µL of the DNA and PLUS™ Reagent dilution into each well across the row corresponding to the label on the tube. Mix gently by slowly pipetting up and down 3 times.

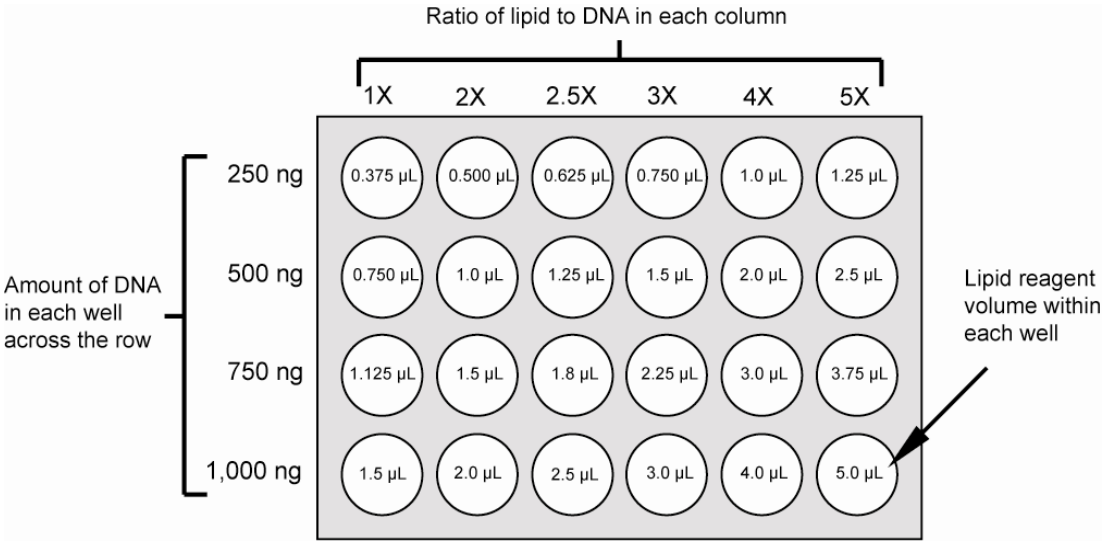
   For example, add 50 µL from tube A into each well across the A row (i.e., A1, A2, A3, A4, A5, and A6. Repeat it for each of the remaining rows of B, C, and D, using the corresponding tubes so that each well contains 100 µL of the appropriate DNA-PLUS™-Lipofectamine™ LTX complex.

6. Incubate the plate containing the DNA-PLUS™-Lipofectamine™ LTX complexes for 30 minutes at room temperature.

7. Transfer the entire 100 µL of the DNA-PLUS™-Lipofectamine™ LTX complex from each well to the appropriate well of your 24-well plate containing your cells in 500 µL of growth medium (see **Example of a 24-well Optimization Plate** below). Mix gently by rocking the plate back and forth.

8. Return your cells to the incubator, and assay for gene expression in 18–48 hours.

Example of a 24-well Optimization Plate



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