

Transfection with Lipofectamine V.1 NDJ 7/21/04

Day before transfection:

Trypsinize and count cells, plating them so they are 50-90% confluent the day of transfection. (10^6 for CHO; 10^5 for HELA)

1. Add 250 μ L serum-free, antibiotic-free media to polystyrene tube.
2. Add 2 μ g DNA.
3. Add 8 μ L of PLUS Reagent, mix, and incubate for 15 minutes at R.T.
4. Meanwhile, add 250 μ L serum-free, antibiotic-free media to separate polystyrene tube. To these, add 12 μ L .
5. Add tube from step 4 to tube from step 3 and incubate at R.T. for 15 minutes.
6. In cell plate, replace media with 2 mL serum-free, antibiotic free media.
7. Add 500 μ L of solution to cell plate, swish, and incubate at 37°C at 5% CO₂ for 3 hours.
8. After 3 hours, add 2.5 mL of 20% serum with antibiotics in order to replace serum to normal levels.
9. Collect lysates 24-48 hours after start of transfection.
10. See Western blot protocol to proceed.