

Nucleofection Quick Guide

100 uL reaction

2 ug DNA

1 - 5 million cells/mL

Do the calculations

1. How many cells go into the reaction? You're in luck. I put 500,000 cells in each flask
2. How much of your DNA solution?

$$\frac{1 \text{ ng}}{\text{uL}} \times \frac{1000 \text{ uL}}{1 \text{ mL}} \times \frac{1 \text{ ug}}{1000\text{ng}} = \frac{1 \text{ ug}}{1 \text{ mL}}$$

$$\frac{100 \text{ ug}}{\text{mL}} \times V \text{ mL} = 2 \text{ ug}$$

$$\frac{2 \text{ ug}}{100 \text{ ug/mL}} = 0.02 \text{ mL}$$

Get everything ready

- New flask for nucleofected cells, labeled:
 - cell type
 - transfected gene
 - date
 - passage #
 - your initials
- Coverslip-bottom dish, also labeled
- Tube of F10 Hams, labeled with your initials
- Warm PBS, labeled with your initials
- Trypsin (shared)
- Mirus reagent (shared)
- Sterile 2 mL tubes
- Sterile 1.5 mL tubes
- Pipetters and tips
- 5 mL serological pipets
- pipet gun
- cuvette
- dropper
- waste beaker
- spray bottle of ethanol

Protocol – Keep everything sterile!

1. Spray all surfaces. Spray everything that goes into the hood.
2. Label new flask to receive nucleofected cells
3. 5 mL F10 Hams into flask, 2.5 mL into coverslip dish, move into incubator
4. 100 uL Mirus/DNA solution in 1.5 mL tube, into incubator
5. Take flask of cells out of incubator, label with your initials
6. Use 5 mL serological pipet to remove medium
7. Put ~ 1 mL warm PBS into flask (sterile transfer pipet or Pasteur pipet)
8. rock to rinse, remove PBS
9. Add ~0.5 mL trypsin (KEEP STERILE), rock to coat cells, put in incubator
10. Incubate 3 minutes
11. Check that cells are in suspension
12. Add 1 mL cold F10 Hams (KEEP STERILE) to stop the reaction.
13. Mix with transfer pipet
14. Transfer ALL to sterile 2 mL tube
15. Spin 500 xG 3 minutes
16. (BACK TO BSC) Remove supernate
17. Resuspend in Mirus/DNA solution
18. Put ALL into sterile cuvette, close
19. Cuvette to Nucleofector
20. Program X001
21. Press the button.
22. (BACK TO BSC) Use special dropper to transfer one drop to the coverslip dish, the rest to the waiting flask.
23. Done!