

Lipofectamine 2000 Transfection of HEK293 cells

Mike Smith December 2002

Cells: One 80-90% confluent T-25 of cells split into a 24 well plate (1ml/well) the day prior to transfection. **Important:** media must be antibiotic-free (that includes zeocin, blasticidin, hygromycin)

DNA: mix DNA at a ratio of 400ng DNA : 1 μ l Lipofectamine 2000
25 μ l DNA + 25 μ l diluted Lipofectamine

For 6 wells you will need:

600 ng DNA in 37.5 μ l serum-free media (SFM)
1.5 μ l lipofectamine in 36 μ l SFM

1. Mix lipofectamine 2000 + SFM, incubate 5' at RT
2. Mix DNA + lipofectamine, incubate 20' at RT
3. Add 525 μ l SFM, mix gently
4. Add 100 μ l mixture per well, return to 37^o incubator
 - a. No need to replace media
5. Change media gently after 24h
6. Stimulate as necessary

To harvest:

Gently dump media out of plate
Blot plate on paper towels
Lyse cells with 100 μ l passive lysis buffer