LIPOFECTAMINE 2000 TRANSFECTION PROTOCOL - 10CM PLATES

Reagents: 10% FBS in OptiMEM, 1x PBS, OptiMEM, Lipofectamine 2000

Protocol:

1. Thaw FBS. Make sufficient 10% FBS in **Opti-MEM** media (**w/o** antibiotics) sterile filter
2. Aspirate medium off cells
3. Add 2-3 ml PBS to wash
4. Aspirate off PBS
5. Add 8 ml of 10% FBS/ Opti-MEM media /plate
6. Incubate 30min at 37°C while mixing following components in hood (see spreadsheet for amounts, this example is for one 10cm plate):
    a. A Tubes: Add selected DNA, and Q.S. w/ Opti-MEM to 100µl (VSV-G if a viral transfection)
    b. B Tubes: Add LF2000 and Q.S. with Opti-MEM to 100µl
    c. Incubate in hood for 5 min
7. Add Component B to A by gently bubble mixing
8. Incubate A/B complexes at RT for 30 min
9. Drip 200µl A/B complex mixture onto cells respectively
10. Gently mix media on cells via figure 8 pattern and back and forth motions
11. Incubated 37° for 6 hours
12. Remove Opti-MEM media containing A/B complexes and discard
13. Add fresh 8ml/ plate of 10% FBS/DMEM with antibiotics

    N.B: if viral transfection Add 12ml 10%FBS in DMEM and 15mM hepes(add 700 ul 0.25 M hepes per 12 ml media **or** make a 1:17 dil'n of hepes stock).Also after 72 hr collection of viral supernatant

Culture Vessel	Surface Area per well (cm^2)	Vol. Plating Media	DNA (ug)	Lipo 2000 (µL)
96-well	0.3	100µl	0.2	0.5
24-well	2	500µl	0.8	2.0
12-well	4	1 ml	1.6	4.0
6-well	10	2ml	4.0	10
60-mm	20	5ml	8.0	20
10-cm	60	15ml	24	60