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## Transfection Medium Protocol for Co-Transfection

Note: This protocol can also be used with ESF 921 or ESF AF instead of Transfection Medium. Efficiencies will be decreased and overall efficiency will be impacted by the purity of the transfer vector.

1. Seed  $2 \times 10^6$  cells per well of 6 well plate. Allow cells to attach for 30 minutes. Alternatively seed  $2 \times 10^6$  cells per well of 24 well deep well block in a very small volume (approx 200 ul). This can be done by growing cells up to  $10^7$ /ml or by centrifuging at 1000 rpm for 5 min and resuspending.
2. Warm Transfection Medium and Lipofection reagent of your choice to room temperature
3. Mix the following:  
  
Solution A: 2 ug recombinant transfer vector  
.5 ug linearized viral DNA  
100 ul Transfection Media  
  
Solution B: 6 ul Lipofection reagent  
100 ul Transfection Medium
4. combine solutions A and B, mix gently, and incubate at room temperature for 30 minutes
5. After incubation add 800 ul Transfection Medium to tubes from step 4, mix gently, add dropwise onto cells.
6. Incubate at 27 degrees C for 4-5 hours, shaking if using deep well block. rpm varies by incubator so adjust around 270 rpm.
7. At the end of incubation bring volume up to 4 ml with 3ml ESF 921 or ESF AF. Incubate for 4-5 days.
8. Collect supernatant 4-5 days post transfection.
9. Efficiency of transfection can be assessed by staining cells for gp64 expression. Because of increased efficiency, amplification protocols may need to be modified either by using less P0 for the amplification or by harvesting the P1 earlier.

