

## TRYPsinIZATION TRANSFECTION PROTOCOL

### **Solutions:**

2 X BBS: 50mM BES pH 6.95 (N,N-bis[2-hydroxyethyl]-2-aminoethanesulfonic acid)  
280mM NaCl  
1.5 mM Na<sub>2</sub> HPO<sub>4</sub>

To prepare 100 ml:

1.066 g BES

1.624 g NaCl

Adjust pH to 6.95 and filter sterilize. Recheck pH – it can change as a result of filtration and it is very important that the pH is 6.95.

### **Procedure:**

1. Plate cells out the day before at  $3 \times 10^6$  cells/plate (for HeLa cells). I have observed that if cells are split from confluent plates at the time of exposure to the ppt., they do not survive the treatment. Grow overnight at regular CO<sub>2</sub> level (5-7.5%).
2. The greatest number of plates I have ever combined to be exposed onto one precipitate is 8-10. If more than this, I would recommend dividing into two sets.
3. Wash cells in PBS and trypsinize with 1 ml 1 X trypsin per plate-use freshly made trypsin (about 5 min. at 37°C). Watch closely to be sure that cells are not over-trypsinized. They should be loose enough to be easily tapped off the plate, but not completely released, nor should you have to pipet vigorously to release them from the plate. Quench with 4 mls DME + 10% C.S. + P/S per plate. It is important to treat the cells with trypsin for the optimum time and to keep this consistent from one set to the next.
4. Meanwhile, as trypsin is working, prepare the precipitate. I prepare the DNA mixture and adjust to 0.25 M CaCl<sub>2</sub> ahead of time for all the precipitates. Then at this step, just add the 2 X BBS. The amount of DNA per plate can vary from 15-25 µg. Whatever amount of DNA you decide to use, adjust the final volume of the transfection cocktail so that the DNA conc. will be 20µg/ml.  
example: 20µg DNA per plate  
8 plates  
T160µg DNA  
To achieve a 20µg/ml conc. of DNA, you will need an 8 ml ppt. Add DNA solution to 0.25 M CaCl<sub>2</sub> in a final volume of 4 ml.  
At step 4, add 4 ml 2 x BBS.  
Mix 2 X BBS with DNA/0.25 M CaCl<sub>2</sub> by pipetting up and down gently (no air bubbles) - cap and let stand 10-15 min. at room temp.
5. When cells are trypsinized, quench and pool into a 50 ml Corning tube. Spin cells down for ~1 min or less at setting 4 - whatever it takes to spin cells down gently. Do this step right before the precipitate is ready. (at the end of the 10-15 min r.t. incub)

6. Pull off media and discard. Using a pasteur pipet, pipet the entire transfection cocktail onto the cells, then gently resuspend cells by pipetting up and down with a pasteur pipet and rubber bulb. Let cells and ppt. stand for 15 at room temperature. About 2-3 times during this period, gently swirl the cells to resuspend them in the ppt. Be gentle.
7. Prepare DME + 10% calf serum + P/S + 6.25 mM CaCl<sub>2</sub> + 0.05 X BBS in sufficient volume for 10 ml per plate. Make this up fresh at the time of transfection (do not store) and be sure to use DME that is quite red, not pink. If pH has become basic you will get a ppt when you add the CaCl<sub>2</sub> + BBS to the media and it will ruin the whole experiment. Keep lid tightly capped between use (Note: cells survive extremely poorly if serum is less than 10%)  
for 10 ml:      10 ml DME + 10% c.s. + P/S  
                         31.25 μl 2 M CaCl<sub>2</sub>  
                         250 μl 2 X BBS
8. After cells + ppt have stood together for 15 min., add DME + 10% c.s. + P/S + 6.25 mM CaCl<sub>2</sub> + 0.05 X BBS in a sufficient quantity to plate out 10 ml per plate, that is, for 8 plates in above example the ppt was 8 mls:  
-add 40 ml DME + 10% c.s./CaCl<sub>2</sub>/BBS  
-pipet up and down thoroughly with a long-tipped 25 μl pipet.  
Place 6 ml each onto 8 plates, where you have placed 4 ml of DME/10% c.s./CaCl<sub>2</sub>/BBS. Tilt plates gently to distribute cells evenly.
9. Place at 35-37°C at 3% CO<sub>2</sub> for 14-16 hours.
10. Wash 2 X with DME + P/S (5 ml per plate) then replace with DME + 10% c.s. + P/S, harvest 24-48 hrs later. If starvation of cells is desired (or lowering of endog. HSP70 levels) replace with DME + P/S only + serum stim or Ad5 induction can be conducted 48 hrs later.

**Reference:**

Combined *Chu + Sharp method (Gene 13, 197-202, 1981) (trypsinization of cells before exposure to ppt)* and *Chen & Okayama low CO<sub>2</sub> method (MCB 7, 2745-2752, 1987).*